ANATOMY AND PHYSIOLOGY OF HAIR RECEPTORS IN THE STATOCYST OF THE CRAB, SCYLLA SERRATA.

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STATEMENT

I declare that all original work presented in this thesis is my own except where otherwise stated.

P. A. Jenn

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SUMMARY

Previous studies have described the canalicular shape of the statocyst of S. serrata and the presence within the statocyst of four different hair types. It has further been shown that the thread hairs are sensitive to angular acceleration. In this thesis the physiological responses of the thread hairs were recorded at the single unit level and the ultrastructural innervation investigated, in a bid to explain the behaviour of the receptors.

There are two bipolar neurons ~~nerves~~ to each thread hair and the connection between the two dendrites ~~nerves~~ and the hair is via a scolopidial structure and a microtubular rod. The insertion of this rod at the hair base resembles that described in certain insect mechanoreceptors. The possible transduction mechanism is discussed and compared to that proposed for other similar receptors.

The hairs are roughly divided into an upper and lower group and they are differentially sensitive to angular accelerations, the upper group being sensitive to yawing movements of the animal and the lower group to pitching or rolling movements.

A single thread hair unit shows a spontaneous impulse frequency that increases or decreases if the hair is forced to bend. During sinusoidal oscillation over a certain frequency range the impulse frequency follows the angular velocity. During constant velocity rotation the impulse frequency is constant unless the rotation is prolonged, when a return to resting level occurs.

The thread hairs can be observed directly to be affected by gravity. This is also reflected in the spontaneous nervous activity of the hairs and in the response to angular

acceleration.

The statocyst and the vertebrate semicircular canal are compared in their roles as angular accelerometers.

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CHAPTER ONE

An Introduction

Until about the beginning of this century, the statocysts of decapod crustaceans were regarded as auditory organs and referred to as otocysts. Some of the evidence cited in support of this conclusion was spectacular indeed, such as that of Aelianus (1784) who claimed that the fishermen of his time caught Pagurus by means of music.

In 1843, Farre published an account of the "vestibular sac" of the lobster and crayfish but reported that he had been unable to find such a structure in any of the brachyuran decapods he had studied.

Hensen, in 1863, published a long, detailed account of the otocyst in many crustaceans, including brachyurans. He divined some similarity between the brachyuran otocyst and the vertebrate semicircular canals, but he was so convinced of the acoustic function of the organ that he tried to apply the names of acoustic components of the vertebrate ear to various structures within the otocyst.

In 1887, Delage removed the organs from three different decapod species and observed that the animals lost their orientation, being, for instance, unable to remain upright while swimming. He was the first to demonstrate the equilibrium function of the otocyst but he still thought that it had an acoustic function as well.

In 1893, Kreidl performed his now famous experiment of inducing a newly moulted Palaemonetes to use iron filings as the new otoliths. He was then able to dictate the position assumed by the animal by means of a magnet held close to the otocyst. His conclusion was that the otocyst was purely an equilibrium organ, with no acoustic function whatever, and that it should, therefore, be called a "statocyst" rather than an

otocyst. Experiments by Clark (1896) supported this view.

Prentiss (1901) reviewed all the previous work on the organ, paying special attention to Hensen. He also performed some experiments of his own in a bid to clarify the confusion surrounding the function of the organ. By repeating the experiments and the results of Beer (1898, 1899), Prentiss demonstrated conclusively that there is no true sense of hearing in decapod crustaceans. There is no sensitivity to airborne vibrations and what sensitivity there is to water-or substrate-borne vibrations is due to low frequency stimulation of tactile receptors, many of which occur on the antennae.

Prentiss conceded a superficial structural similarity between the brachyuran otocyst and the vertebrate semicircular canals, as had been described by Hensen. However, he asserted that, since the compartments were not canalicular, were in free communication with each other, and were not arranged, relative to each other, in any way that could be of functional importance, there was no reason for comparing the otocyst with the semicircular canals. He concluded that the superficial structural resemblance was purely coincidental and was functional in the otocyst only in as much as it provided better attachment for muscles within the basal segment.

In the main, Prentiss found Hensen's anatomical work reliable. For example, Hensen's classification of the sensory hairs into thread hairs, hook hairs and group hairs is still valid today, with only slight modification. Some of his sketches were very accurate, especially his drawing of the base of the thread hair and his description of it as a "spherical membrane in a cup-like depression".

There was one subject on which several earlier

workers, including Hensen, did not agree. This concerned the peripheral nerve terminations in the sensory hairs and Prentiss attempted to clarify the situation.

The confusion over the nature of the innervation of the sensory hairs seems to have arisen from the fact that, in many cases, all the sensory hairs associated with the antennule were studied together. Prentiss demonstrated that there were fundamental differences between mechanosensory and olfactory hairs in that the latter were invariably each supplied with a number of dendrites whereas the former were supplied with only one. In addition, the dendrites travelled up inside the shaft of the olfactory hairs, ending at or near the tip, whereas the dendrite supplying the mechanosensory hair terminated at the base of the hair. Both of these facts about mechanosensory hairs may now be in dispute, in some cases at least, but it was a progressive step in the investigation of these systems at the turn of the century.

One observation of Hensen's with which Prentiss fully concurred was that brachyuran decapods lacked otoliths. Prentiss actually only examined one or two brachyurans but it is remarkable that workers before and after him were also unable to find any otoliths in crabs. In fact, it was not until 1956 that they were first reported (Dijkgraaf, 1956).

For Prentiss, the failure to find otoliths resulted in his completely misunderstanding the functions of the various sensory hairs within the otocyst. Because all the other decapods, and even larval brachyurans, had hook hairs surmounted by otoliths, he concluded that the hook hairs in Carcinus must be functionless vestiges, because no otoliths were to be found. The group hairs were a mystery then, as now, so Prentiss

reasoned that the thread hairs must be the most important sensory hairs in the otocyst. He thought that they had taken over the function of gravity reception, and suggested a way in which they might do this. However, if he had realised the significance of some of his experimental results, Prentiss may well have discovered, 50 years ahead of Dijkgraaf, the true function of the thread hairs.

In the next 50 years, apart from isolated reports such as those of Kinzig (1919) and Debaisieux (1947), very little work seems to have been done on the crustacean equilibrium organ. By the early fifties, when Schöne and Dijkgraaf began publishing on the subject, the term "statocyst" had become the accepted term for this structure.

Schöne (1951, 1954, 1957, 1967) concentrated on the statolith receptors and performed detailed behavioural experiments on the mysid, Palaemonetes and the lobster, Panulirus argus.

Dijkgraaf's (1956) immediate contribution to our understanding of the crab statocyst was threefold. Primarily, he demonstrated incontrovertibly that there were granules or liths present in the statocysts of both Carcinus and Maja verrucosa. Since the organ was now called a statocyst, he called the collection of granules a "statolith". Secondly, he showed that the statolith was associated with a particular group of small hooked hairs which were arranged in a roughly circular pattern, their hooks all directed in towards and touching the statolith. This patch of hairs was in the ventral part of the statocyst and Dijkgraaf called them "statolith hairs" and renamed Hensen's hook hairs as "free hook hairs",

because they do not bear a statolith. His third major contribution concerned the thread hairs. By selectively inactivating them or inactivating all hairs except them, he was able to show that they were the hairs responsible for the detection of rotation of the animal, especially about a vertical axis. Because of this fact alone, i.e. on a purely functional basis, Dijkgraaf compared the thread hairs to the semicircular canals of the vertebrate labyrinth.

Dijkgraaf studied the physiology of the brachyuran statocyst in terms of the contribution made to the behaviour of the whole animal by the different groups of hairs. This he did mainly by precise extirpation of different parts of the system and careful observation; he did not record the nervous output of any of the sensory hairs within the statocyst.

Cohen (1955, 1960) studied the lobster, Homarus, and has presented by far the most detailed physiological study of the decapod statocyst. No comparable study exists, however, on a brachyuran statocyst and, though they doubtless share a common ancestry, the statocysts of brachyurans and those of other decapods are considerably different in structure.

For example, the macruran statocyst is a simple sac with one major invagination forming a sensory cushion surmounted by sensory hairs. There is no obvious canal structure to the statocyst and a large statolith, sitting on the sensory cushion, fills a large proportion of the volume of the statocyst.

The brachyuran statocyst usually has a much more complex shape, being subdivided into compartments. The compartments are all in communication with one another, often along well-defined channels or canals, but the compartmentation appears to be an attempt at separating the sensory areas responsible

for different types of equilibrium function. As well as separation, some of the structural developments allow a measure of specialisation in the different sensory areas, which enhances the sensitivity to, and discrimination of, disturbances in the equilibrium of the animal.

There are possible evolutionary implications of this trend towards subdivision of the equilibrium organ especially, perhaps, as the most primitive of the vertebrates, the cyclostome, also has only two semi-circular canals instead of the usual three found in the higher vertebrates. However, it has also been suggested (Sandeman, 1973) that the different forms of statocyst in macrurans and brachyurans may be correlated with the different body forms of these two groups. Thus, macrurans, with their elongated body, are presumed to be predominantly subject to rolling movements. The most useful equilibrium organ, then, would be one which was predominantly sensitive to the direction of the gravitational force, i.e. a statolith system. A highly developed lith system is present in macrurans. Brachyurans, on the other hand, with their more rounded bodies, are not particularly unstable about any axis, but they are continually engaged in yawing and pitching movements. In this situation, an equilibrium system capable of detecting the direction and magnitude of angular rotation would be required if the animal was to remain genuinely in equilibrium.

Sandeman (1976) has recently made the further point that, when swimming in open water, ~~swimming~~ crabs do not have the benefit of any proprioceptive input from their legs and receive little information from the visual system, ~~when swimming in open water~~, so that a well-developed, three-dimensional, dynamic equilibrium organ would be essential for the animal's stability.

The fact that the statolith was not found in brachyurans until approximately 20 years ago is an indication of its minute size relative to the statocyst and this may in turn reflect the relative lack of importance in the brachyuran statocyst. Dijkgraaf's experiments, on the other hand, reveal how very sensitive such a statocyst is to angular rotation, this sensitivity being mediated by the delicate, free-standing thread hairs.

Sandeman and Okajima studied the Australian mudcrab, Scylla serrata and, in 1972, published an account of the anatomy, innervation and basic physiology of the statocyst. In the first electrophysiological recordings from a crab statocyst, they confirmed that the statolith hairs are position receptors and the thread hairs dynamic receptors, very sensitive to fluid flow within the statocyst. They also showed a high-threshold response to fluid flow in the free hook hairs, confirming the observations of Cohen and Dijkgraaf (1961) in Maja and Carcinus. Further, they explained how the canalicular structure of the statocyst and the arrangement of the thread hairs combined to render the structure especially sensitive to angular rotation about any axis.

Sandeman (1973) elaborated on this latter aspect of the statocyst and Fraser and Sandeman (1975) tested certain predictions about the sensitivity of the statocyst to specific stimuli, using interneurons in the oesophageal connectives as monitors of thread hair activity.

Apart from this, Sandeman's early work with Okajima and later work with Silvey (in press) were both directed primarily towards an understanding of statocyst-induced

compensatory eye movements and the integrations and motor control involved therein. Consequently, no single unit analysis of individual receptors was undertaken and, likewise, the fine structure of the receptors was not investigated.

There has been little progress in the fine anatomy of the sensory hairs of the statocyst since they were first described. Hensen described the innervation of some of the hairs and Kinzig (1919) essentially confirmed Hensen's observations without adding anything new.

Schöne and Steinbrecht (1968) investigated the statolith hairs of the crayfish, Astacus, using the electron microscope and found the structure to be much the same as reported by Kinzig, except for one major detail.

The spherical membrane is heavily sclerotised on one side to form a rigid tooth which acts as the fulcrum about which the shaft of the hair articulates. Below the cuticle, where Kinzig and earlier workers reported a single sense cell to each hair, Schöne and Steinbrecht found three dendrites to each hair. Each is heavily ensheathed and each gives rise to a ciliary structure, which breaks down to a mass of microtubules. The three sensory processes, each consisting of a mass of microtubules, end bluntly and are embedded in the end of a rod-like granular structure, the chorda. This runs along under the cuticle, up through a pore, traverses the lumen of the cask-shaped membrane and inserts firmly at the base of the lingula, a cuticular spine of the hair shaft. No comparable detail is available for the thread hairs of any statocyst.

The aim of this thesis was to investigate in detail the behaviour and electrical responses of the thread hairs, individually and as a group, and to discover the ultra-structural basis for such behaviour.

CHAPTER TWO

Materials and Methods

MATERIAL AND METHODS

The experimental animal was the Queensland Mud Crab, Scylla serrata. Specimens were kept alive in the laboratory for 2-4 weeks on damp hessian in covered plastic bins. Experiments were performed on animals of either sex and ranging in carapace width from 10 to 20 cm.

For both the physiological and the anatomical work the preparatory dissection was essentially the same:

After persuading the animal to autotomise its legs, the animal was held in one hand, ventral side uppermost, while most of the mouthparts and the thin carapace covering the gills were cut away. From the dorsal side, the carapace was cut across the whole width of the animal approximately 2 cm from the anterior edge. By gently pushing forward and down on this anterior strip of carapace, the oesophagus and connectives posterior to the brain were exposed and severed. The remaining muscles and connective tissue linking the two portions of the animal were quickly snipped away and the anterior portion of the animal, after some trimming, was then mounted in a saline bath, ventral side uppermost. The saline used in all experiments was that developed for Carcinus by Pantin (1934).

Connective tissue was carefully cut away to expose the brain and the nerve bundles radiating from it.

The statocysts are located within the enlarged basal segments of the antennules. (Fig. 1). The posterior ventral wall of the cup containing the antennule was cut away and the antennulary nerve traced from the brain into the basal segment of the antennule. (Fig. 2).

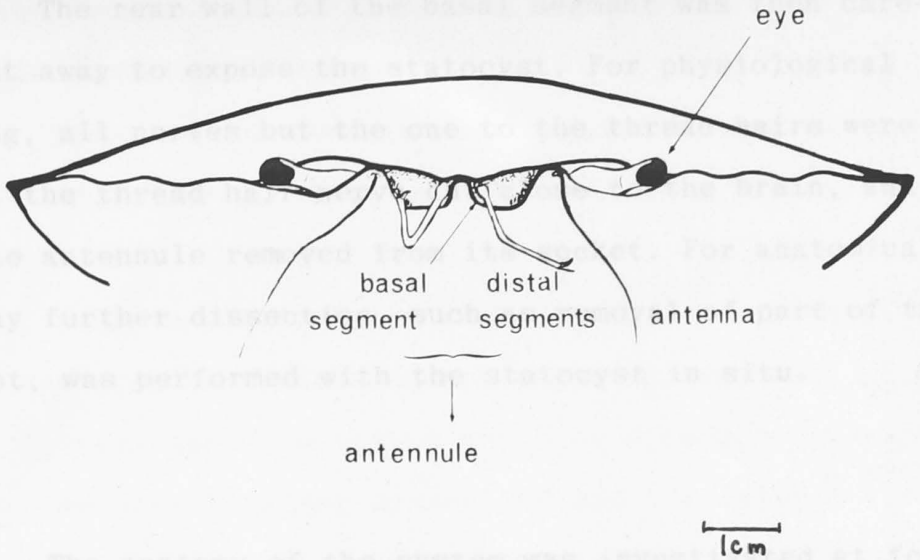


FIG. 1 anterior view of experimental animal

RIGHT LEFT

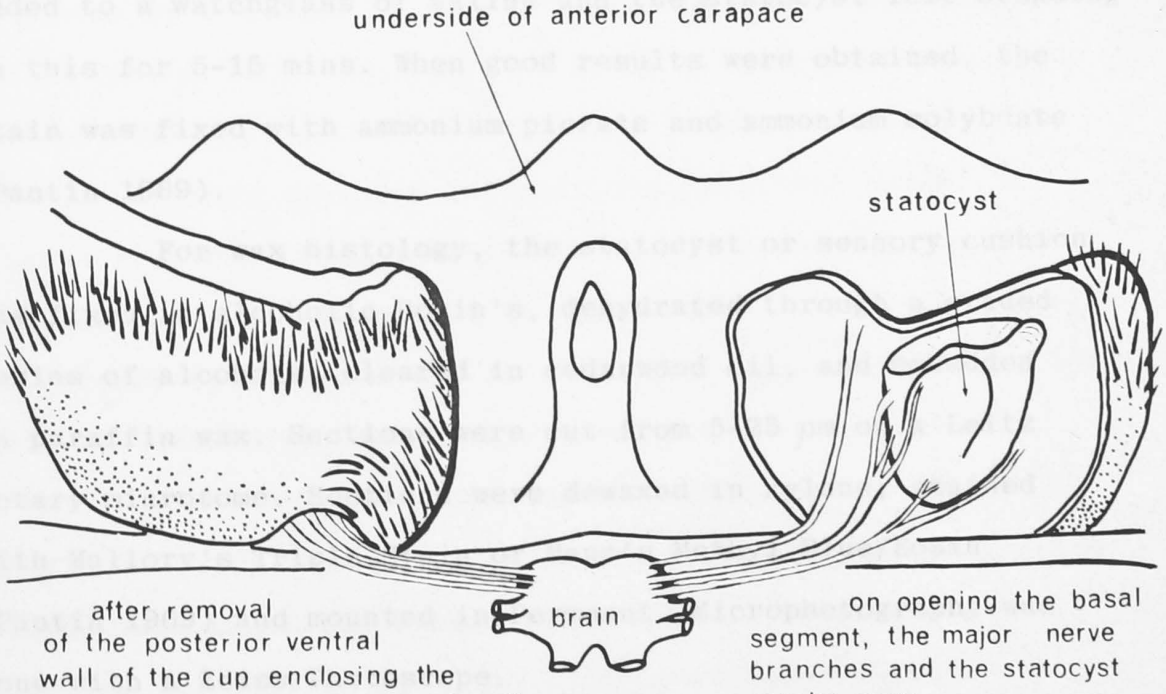


FIG. 2 diagram of ventral dissection

The rear wall of the basal segment was then carefully cut away to expose the statocyst. For physiological recording, all nerves but the one to the thread hairs were removed, the thread hair nerve cut close to the brain, and the whole antennule removed from its socket. For anatomical work, any further dissection, such as removal of part of the statocyst, was performed with the statocyst in situ.

Anatomy

The anatomy of the system was investigated at four different levels; light microscopy of whole mounts and wax sections, and scanning and transmission electron microscopy.

For staining the nerve elements of the intact statocyst, several drops of 1% solution of methylene blue were added to a watchglass of saline and the statocyst left standing in this for 5-15 mins. When good results were obtained, the stain was fixed with ammonium picrate and ammonium molybdate (Pantin 1969).

For wax histology, the statocyst or sensory cushion was fixed in alcoholic Bouin's, dehydrated through a graded series of alcohols, cleared in cedarwood oil, and embedded in paraffin wax. Sections were cut from 5-25 μm on a Leitz rotary microtome. Sections were dewaxed in xylene, stained with Mallory's Triple Stain or Mann's Methyl Blue/Eosin (Pantin 1969) and mounted in Permount. Microphotography was done with a Zeiss Photoscope.

Electron microscopy of marine animals is often difficult, but reasonably good fixation was obtained with two solutions. One was Fahrenbach's (1971) fixative and the other a solution of 5% glutaraldehyde in sea water. The

latter was developed in this laboratory by Dr E.E. Ball (personal communication).

Problems inherent in sectioning the hard cuticle were avoided by using, where possible, moulting animals, in which the cuticle is soft.

Since delicate hairs were the object of study, an attempt was made to minimise harsh physical treatment of the tissue. To this end, the tissue was placed in porous Reichert capsules immediately after dissection and the capsule transferred from saline to fixative to buffer etc. This prevented the tissue from being exposed to the air at any time and reduced the strong fluid flows involved in, say, transferring tissue in a pipette.

Fixation for two hours at room temperature was followed by a couple of short washes in* a buffered solution of 1% Osmium Tetroxide, followed again by several washes in buffer. Dehydration was via a graded ethanol series and the tissue was then embedded in resin via propylene oxide. Two resins were employed, TAAB (TAAB Laboratories, Reading, England) and that developed by Spurr (1969). The only practical difference between the two is that TAAB is relatively non-toxic and this made the embedding procedure easier.

Sectioning was done on a Reichert Om U2 ultramicrotome using both glass and diamond (DuPont) knives. Thick sections (0.5-1.0 μm) were stained with 1% toluidine blue solution for viewing with the light microscope. Silver and gold sections, for use in the electron microscope, were mounted on coated copper slot grids.

For coating, several different solutions were tried and the most successful was a solution of 0.3% Formvar in

* buffer and secondary fixation for one hour in....

Ethylene Dichloride.

The sections were stained for 20-40 minutes in saturated aqueous Uranyl Acetate, followed by 20 minutes in Reynold's Lead Citrate (1963), and examined in a Jeol JEM-100C Electron Microscope, operated at 60-80 Kv.

For scanning electron microscopy, the tissue was carefully dehydrated in a graded acetone series, usually without fixation. Once again, care was taken not to expose the tissue to the air. It was then critical point dried from acetone and vacuum-coated with carbon and gold/palladium. Specimens were viewed on a Hitachi HHS-2R Scanning Electron microscope, operated at 15 Kv.

Physiology

In order to record the responses of the primary sense cells during stimulation of the statocyst, the antennulary nerves were cut close to the brain and the entire antennule removed from the socket.

The distal segments of the antennule were removed and the basal segment mounted on a mound of vaseline in a dish of saline. The orientation of the segment was arranged to coincide with that in the animal. The thread hair nerve was usually teased into fine strands, one of which was then laid over a silver-wire hook electrode or taken up into a fine-tipped suction electrode. The saline level was lowered and a drop of mineral oil prevented desiccation of the nerve.

Responses were amplified with a Devices 3160 amplifier and displayed on an oscilloscope (Tektronix 502A), along with the stimulus, and recorded with a Nihon Kohden oscilloscope camera. In some experiments, a single unit was used to trigger

the oscilloscope, whose output was fed into a ratemeter. The output of the ratemeter was summed and averaged by a Hewlett Packard 5480B Signal Analyser, whose display was then photographed with a Polaroid camera.

Dynamic stimulation was achieved by mounting the lightly constructed dish directly onto an electromagnetic pen-motor, which was driven by a Servomex LF.51 low frequency waveform generator. This had a wide range of output waveforms but only two were used regularly, sinusoidal voltage oscillation and voltage ramps, which produced sinusoidal oscillation and constant velocity rotation of the dish, respectively. A 90 volt peak-peak output from the generator produced a 10^{o} peak-peak amplitude of rotation of the dish.

Any problems of phase lag between the generator and the motor were obviated by directly monitoring the movement of the dish with a photocell.

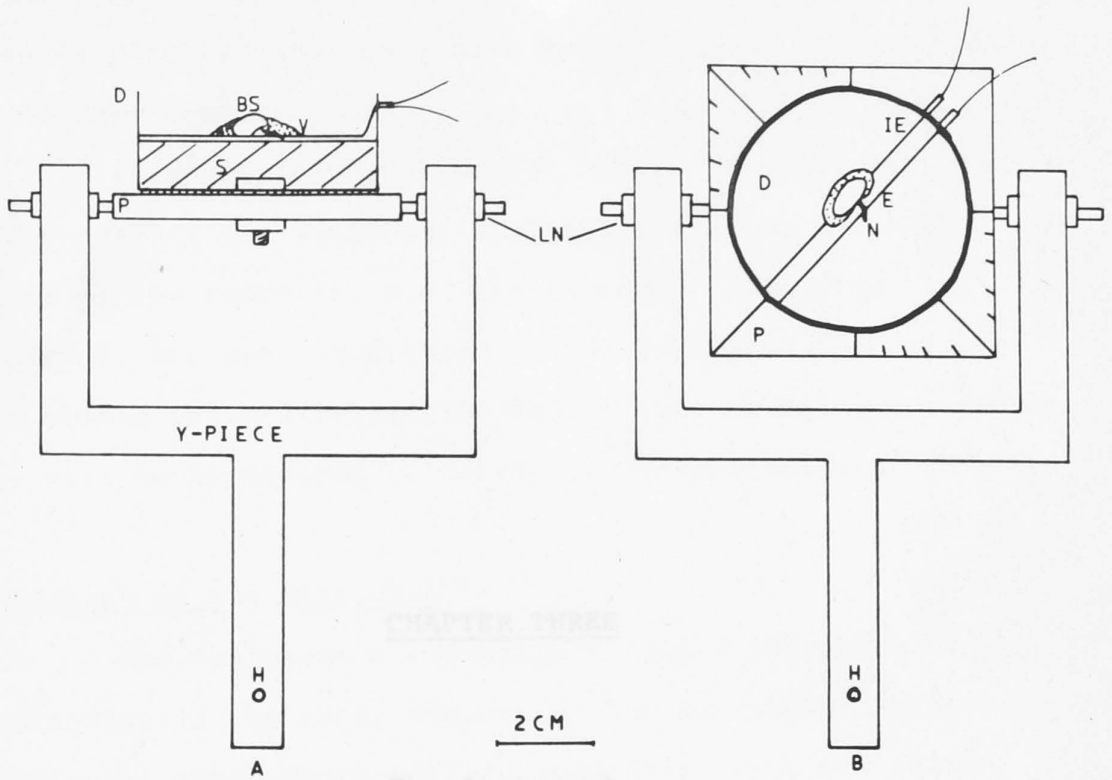
As the responses of the thread hairs to different types of stimulation were being investigated, the stimulating apparatus was assembled in such a way that adjustments could be easily made to, for instance, the plane of rotation of the statocyst. The dish itself was made of several pieces of plexiglass whose positions with respect to each other could be altered. In addition, the pen motor was mounted on a calibrated device so that the plane of stimulation could be set at any position between the horizontal and the vertical (Fig. 3).

Figure 3

Apparatus used to stimulate the statocyst and record the nervous responses.

A plexiglass Y-piece supports on a spindle a plexiglass plate (P), which can be fixed at right angles to the Y-piece (A) or in the same plane as the Y-piece (B) or in any other position by the locking nuts (LN) on the spindle. A perspex dish (D) is bolted fairly tightly to the plexiglass plate. The gap between the dish and the plate contains a layer of thick vacuum grease so that the dish can be rotated to any position on the calibrated plate. The dish is half-filled with Sylgard transparent moulding rubber (S). The basal segment (BS), containing the statocyst, is mounted in a mound of vaseline (V) over the indifferent electrode (IE) and the appropriate nerve (N) draped over the active electrode (E).

Lower diagram shows the pen-motor mounted on a calibrated disc. The wand moves only in and out of the page and supports the Y-piece by inserting in the hole in the stem. For yaw movements, the dish arrangement shown in (A) is mounted on the motor in the position shown. For pitch or roll movements, the motor is rotated counterclockwise through 90° and the dish araangement shown in (B) is mounted on it.


D
BS
V
S
P
LN
Y-PIECE
H
O
A
2CM
IE
D
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N
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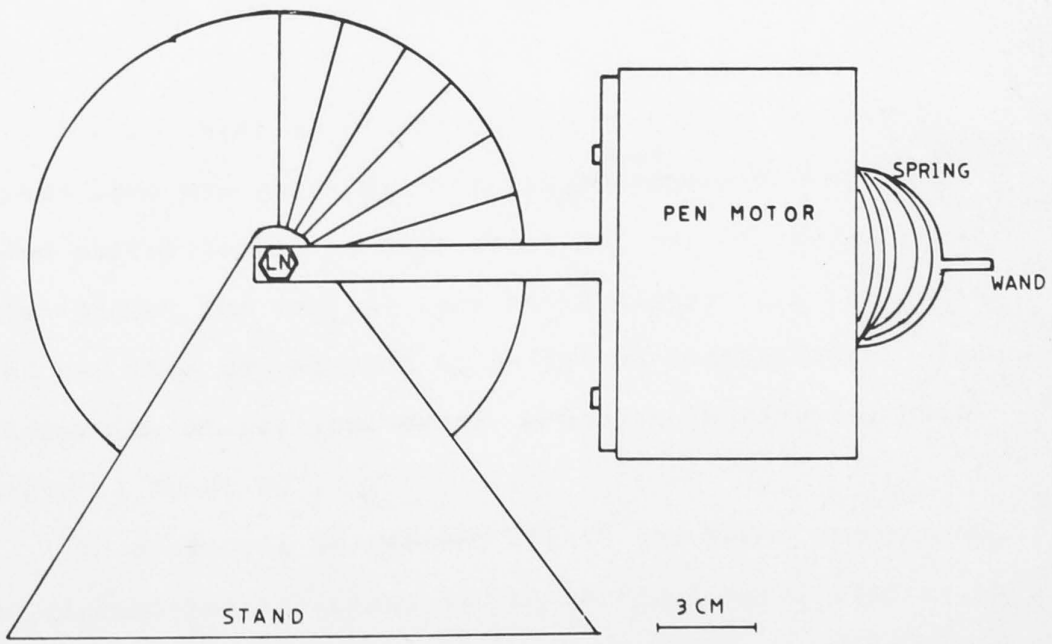

LN
PEN MOTOR
SPRING
WAND
STAND
3CM

CHAPTER THREE

The Statocyst

Introduction

The decapod statocyst is basically a fluid-filled cuticular chamber, extensively innervated by branches of the antennulary nerve and containing several types of small hairs, most of them sensory.

This chapter describes in detail the internal architecture of the statocyst of Scylla. It also contains details of the composition of the enclosed fluid, the statolymph, and describes three of the four hair types to be found within the statocyst. The fourth type of hair, the thread hair, will be considered in detail in three subsequent chapters.

Development of the Statocyst

The statocyst has developed from a cuticular invagination in the basal segment of the antennule. Secondary asymmetrical compression of this cuticular structure causes further infolding. The result is the formation of two incomplete toroids which meet almost at right angles in a common canal (Fig. 1).

The orientation of the statocyst within the basal segment has been described by Sandeman and Okajima, (1972). The dorsal toroid is only about 20^{o} out of the horizontal plane, the lateral edge being higher than the medial edge, and can thus be referred to as the horizontal canal. The vertical toroid, or vertical canal, meets the horizontal canal at an angle of about 90^{o}.

The statocyst is twisted within the basal segment so that the circumferential plane of the vertical canal lies at an angle of 45^{o} to the longitudinal axis of the animal. This is important with regard to the sensitivity of the system to

Figure 1

The possible development of a two-canal structure from a single invagination of the exoskeleton. A round vesicle, (1) sealed at its point of invagination, is compressed laterally and dorso-ventrally as shown by the arrows (2) and the result is a pair of toroids (3) joined in a common arm and oriented so as to form the horizontal and vertical semicircular canals.

(Redrawn from Sandeman, 1973.)

Figure 2

The orientation of the statocyst canals to the vertical, longitudinal and transverse axes as viewed from the back of the animal. The plane of the vertical canal is at 45° to the longitudinal and transverse axes of the animal and is tilted 20° out of the vertical. The posterior arm of the horizontal canal lies at 90° to the vertical canal while the anterior arms make an angle of 95° with each other.

(Redrawn from Sandeman, 1973.)

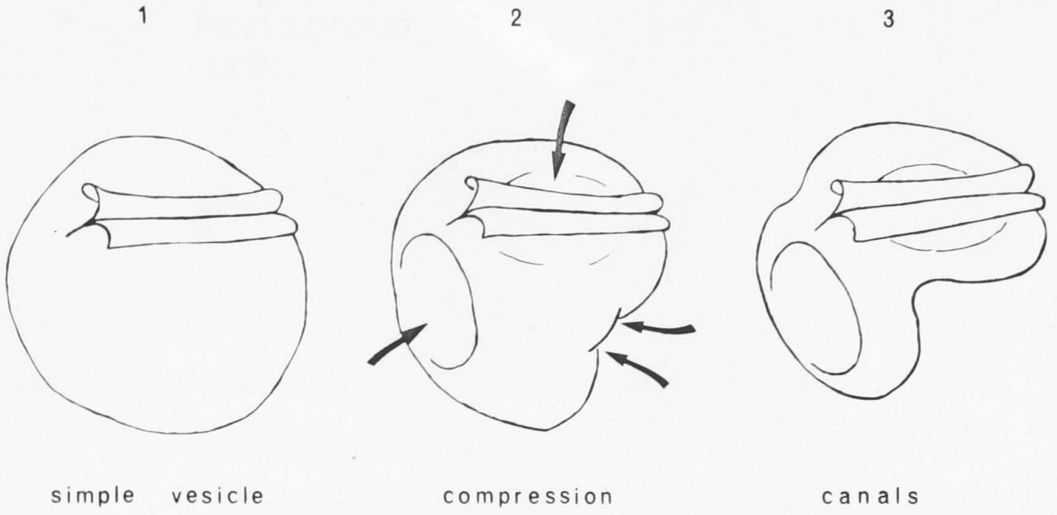

1
2
3
simple vesicle
compression
canals

FIG. 1

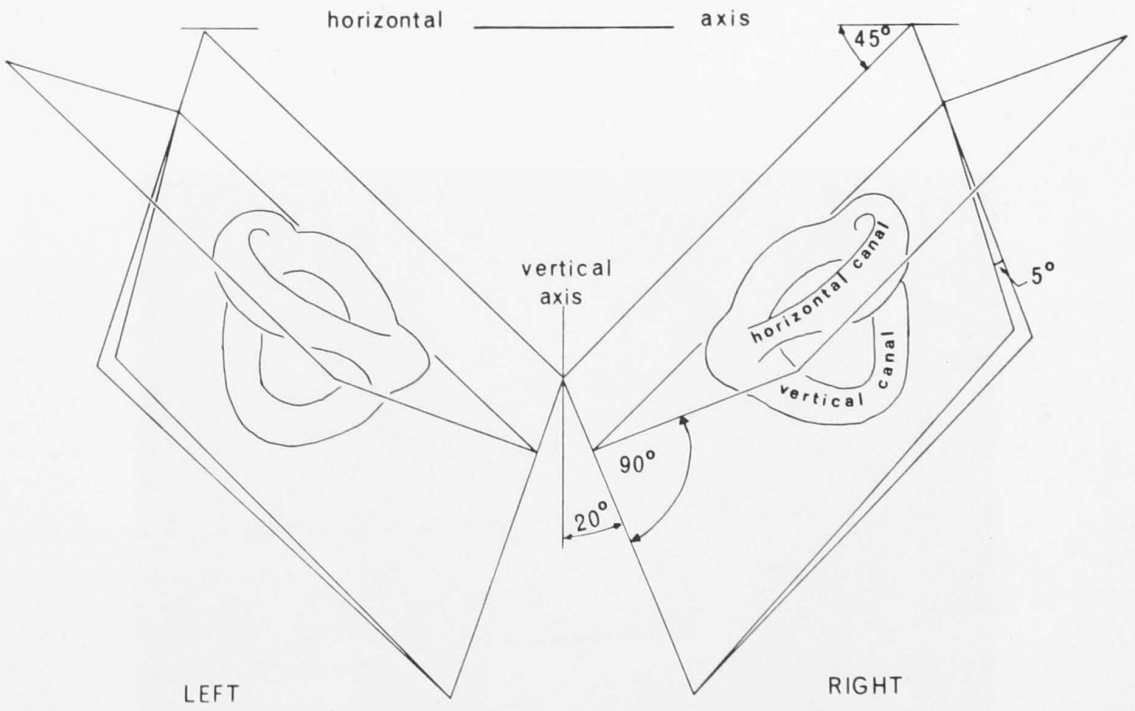
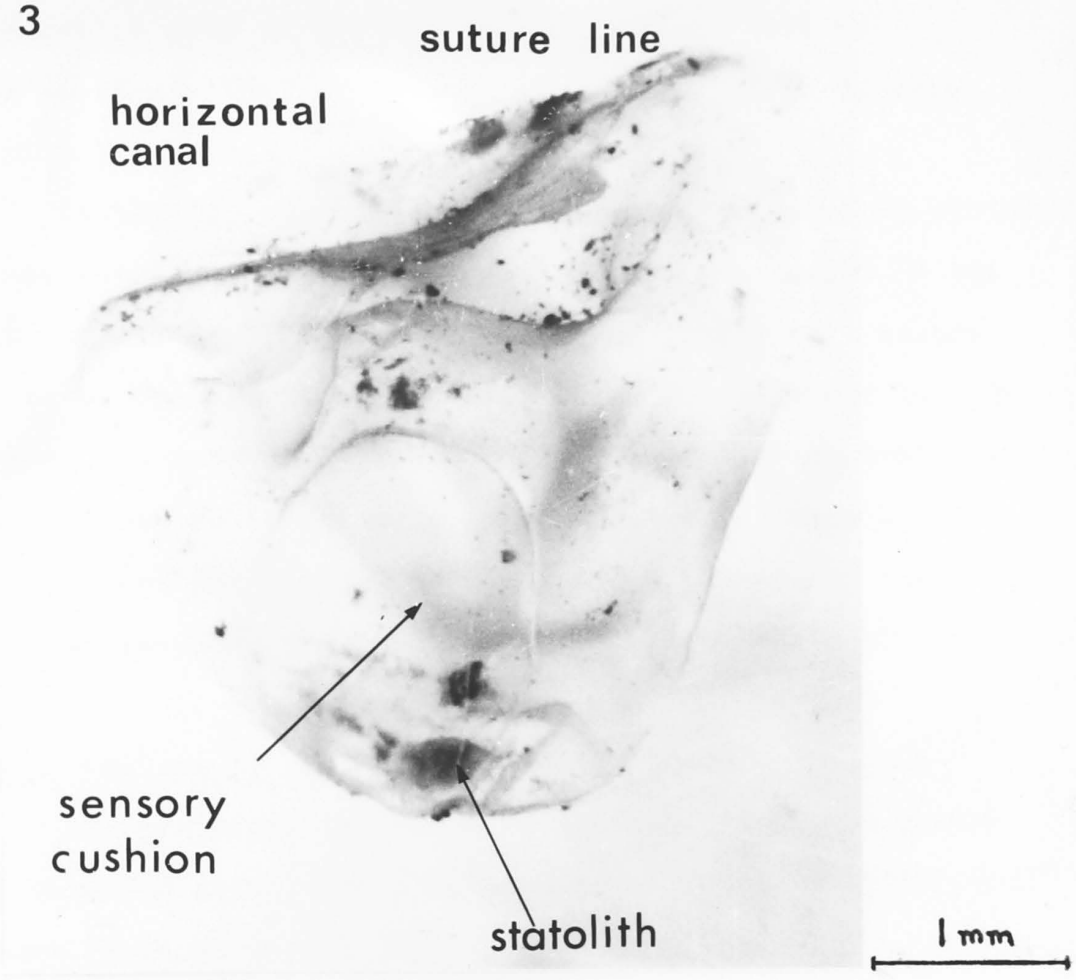
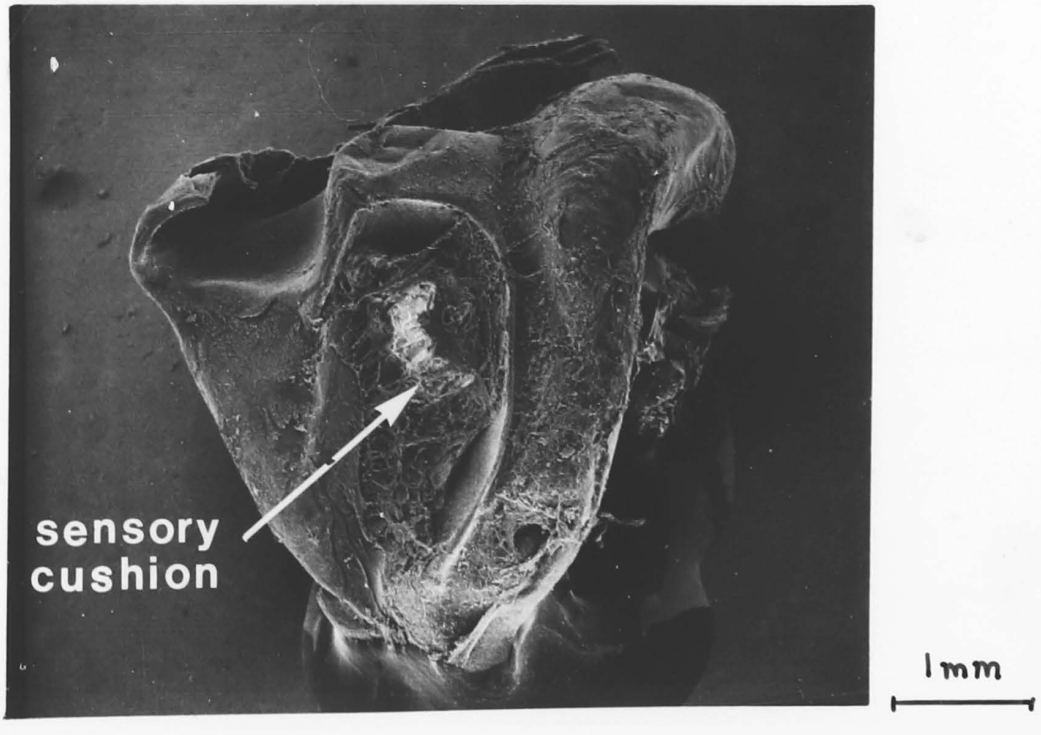

horizontal
axis
45°
vertical
axis
horizontal canal
vertical canal
5°
90°
20°
LEFT
RIGHT

FIG. 2

Figure 3

Photograph of the statocyst floating in saline (upper) and scanning electron micrograph (lower) show the general shape of the statocyst and the suture line running diagonally across the horizontal canal.


suture line
horizontal canal
sensory cushion
statolith
1 mm


sensory cushion
1 mm

displacement in both of the vertical planes and will be referred to later. The arrangement of the pair of statocysts is shown in Figure 2.

In Scylla, the statocyst is open for an unknown period during moulting but appears to be a sealed chamber most of the time, the suture line being quite conspicuous dorsally across the centre of the horizontal canal (Fig. 3). The suture line is attached to the dorsal cuticular wall of the basal segment. In addition, within the rightangle formed by the two canals, part of the statocyst cuticle is thickened and hardened and fused to cuticular apodemes from the wall of the basal segment.

Detailed Structure of the Statocyst

The statocyst increases in size as the animal grows and the observed relationship between animal size and statocyst dimensions is shown in Fig. 3A.

The average experimental animal measured about 13 cm across the carapace and here the statocyst canals have an external diameter of about 3.5 mm. The lumen of both canals measures approximately 0.7 mm in diameter with localised areas of larger or smaller dimensions.

The horizontal canal is a complete toroid in that the dorsal and ventral depressions usually meet and fuse so that a sealed canal runs in a circle around a central pillar of cuticle. The vertical canal, on the other hand, has a much more complex structure. The respective depressions either side of the canal do not meet, although, at their closest point, the gap may be as little as 50 μm. This means that the circumferential canal is not completely sealed. Figure 4 illustrates this difference between the two canals.

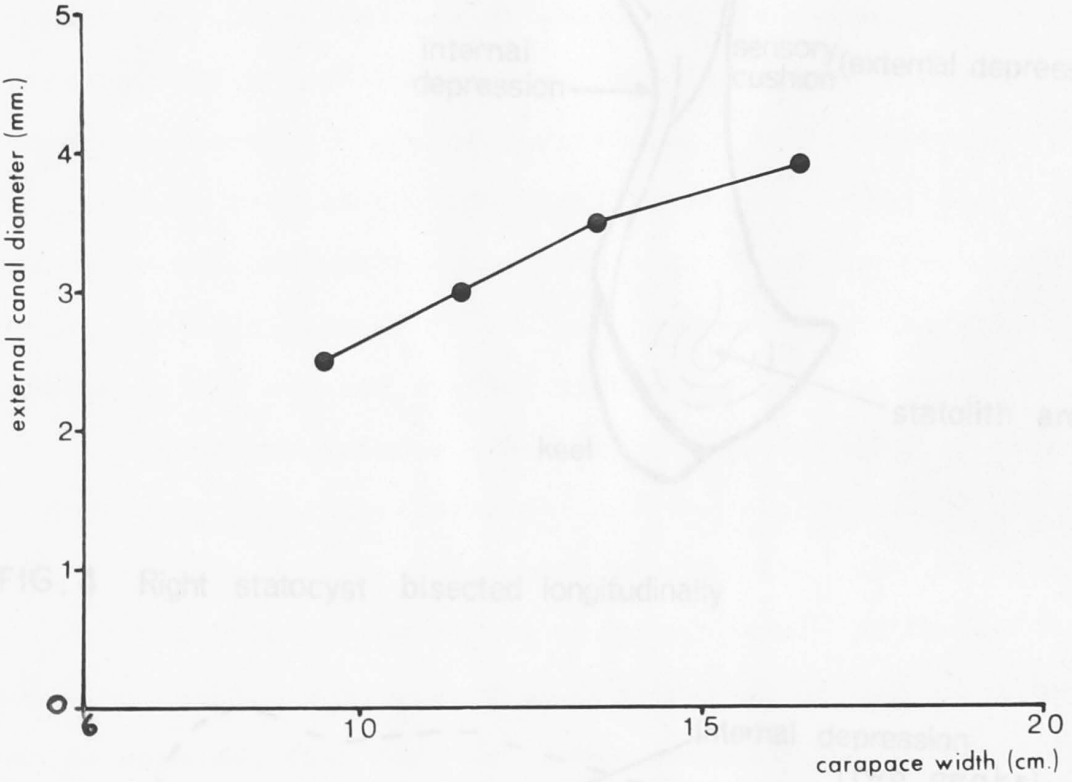


FIG. 3 A Variation in external diameter of statocyst canals with carapace width in Scylla serrata.

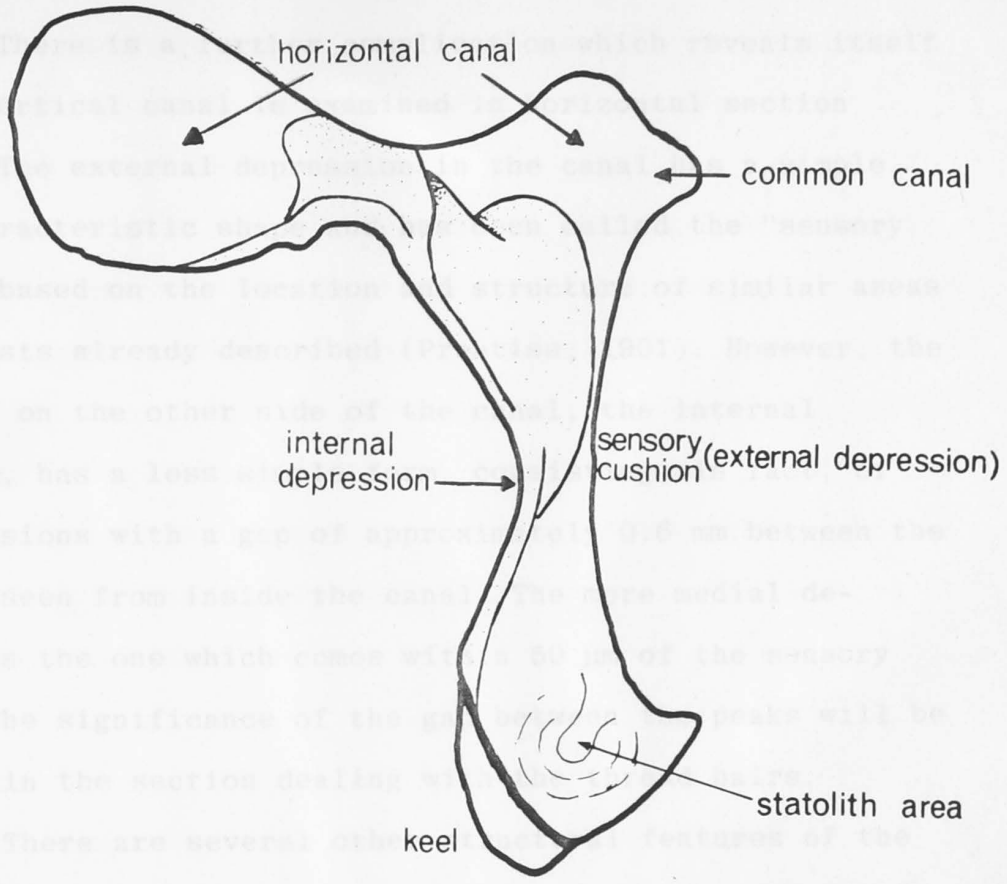


FIG. 4 Right statocyst bisected longitudinally

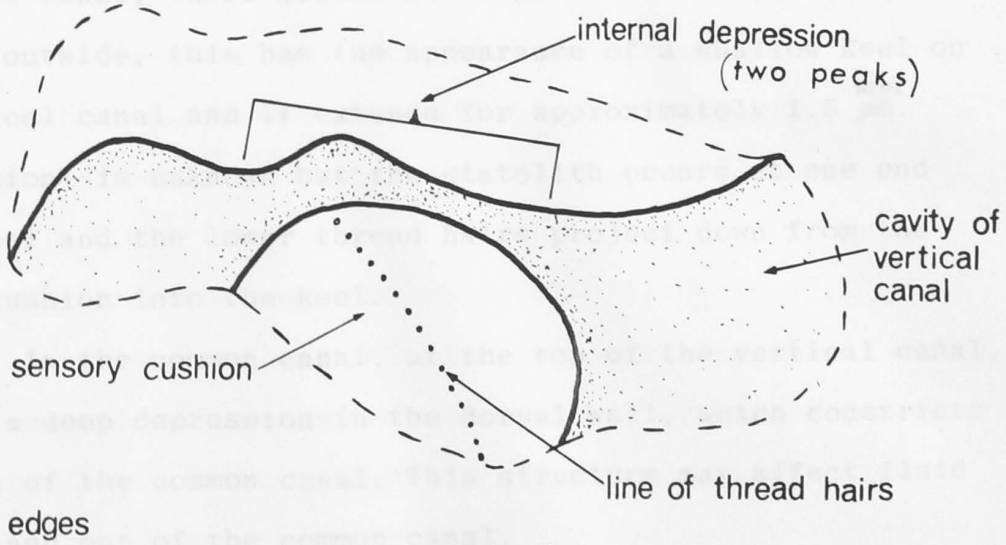


FIG. 5 Right statocyst, horizontal canal removed; view down vertical canal

There is a further complication which reveals itself when the vertical canal is examined in horizontal section (Fig. 5). The external depression in the canal has a simple, though characteristic shape and has been called the "sensory cushion", based on the location and structure of similar areas in statocysts already described (Prentiss, 1901). However, the depression on the other side of the canal, the internal depression, has a less simple form, consisting, in fact, of two depressions with a gap of approximately 0.6 mm between the peaks, as seen from inside the canal. The more medial depression is the one which comes within 50 μm of the sensory cushion. The significance of the gap between the peaks will be described in the section dealing with the thread hairs.

There are several other structural features of the statocyst of Scylla which have not so far been described but which may play a role in the functioning of the organ.

Near the bottom of the vertical canal, in the medial arm of the canal, there occurs an evagination of the outer wall. From the outside, this has the appearance of a shallow keel on the vertical canal and it extends for approximately 1.5 mm. Its function is unknown but the statolith occurs at one end of the keel and the lower thread hairs project down from the sensory cushion into the keel.

In the common canal, at the top of the vertical canal, there is a deep depression in the dorsal wall, which constricts the lumen of the common canal. This structure may affect fluid flows in and out of the common canal.

At this point, it should be emphasised that the statocyst is asymmetrical about a vertical line through the centre of the vertical canal and that the members of a

pair of statocysts are mirror images of each other (Fig. 6).

A third structural feature of the statocyst of Scylla which has not been reported before concerns a patch of "pores" found at each end of the sensory cushion, close to the thread hairs. (~~Fig. 8~~). They have been seen, so far, only in the scanning electron microscope and have been called pores purely on their appearance, with no physiological evidence whatsoever. Holes in the cuticle of a different form have been found in the statolith area and the possible functions of both these and the pores will be discussed later in the chapter, in the section dealing with the statolith.

Statolymph

It was considered important to know as much as possible about the fluid inside the statocyst, the statolymph, because of its essential role in the functioning of the statocyst, especially during angular acceleration. The small volume of fluid available from each statocyst (10 µl approx.) imposed certain restrictions on the type of analysis that was possible, but measurements were obtained of the density, viscosity, and chemical composition of the statolymph.

Density

The density was measured in the following way. A 100 ml beaker was weighed dry, and then with exactly 50 ml distilled water, and then with exactly 50 ml saline. Since the volumes of the fluids were identical, the ratio:weight of saline/weight of water, gives a value for the relative density of the saline, which is 1.02. A 10 µl syringe was weighed dry, then full of distilled water, and then full of saline. On the same principle as above, a value for the relative density

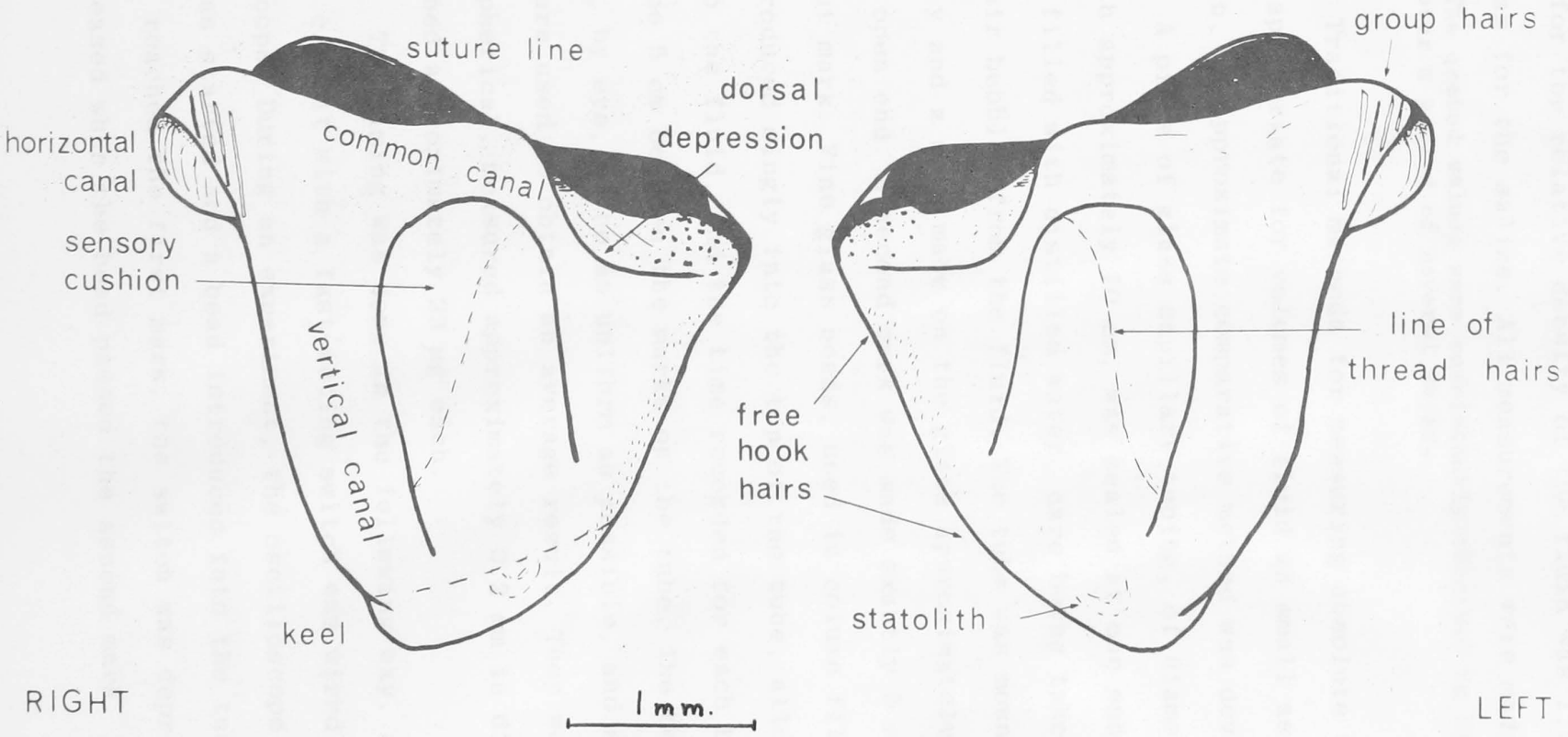


FIG. 6. Diagrammatic view of pair of statocysts from front of animal.

of saline was obtained, which was 1.02. This procedure confirmed the validity of the technique. The final measurement required weighing the syringe full of statolymph. The value obtained for the relative density of the fluid was 1.02, i.e. the same as for the saline. All measurements were made at 21.5°C. The quoted values were consistently obtained in five analyses over a period of several weeks.

Viscosity

Traditional methods for measuring absolute viscosity were not appropriate for volumes of fluid as small as 50 µl, so, once again, an approximate comparative method was developed.

A piece of glass capillary tubing, of diameter 1 mm and length approximately 10 cm, was sealed at one end. The tube was then filled with distilled water, care being taken to exclude air bubbles from the fluid. The tube was mounted vertically and a mark made on the glass approximately 4 cm down from the open end. A second mark was made exactly 5 cm below this first mark. Fine glass beads, used in column filtration, were introduced singly into the top of the tube, allowed to fall into the fluid, and the time recorded for each bead to travel the 5 cm between the marks on the tube. The beads were selected, by eye, to be as uniform as possible, and a large number were used to obtain an average result. They were virtually spherical, measured approximately 0.2 mm in diameter, and weighed approximately 23 µg each.

The timing was done in the following way. A simple 1.5 volt circuit with a fast-acting switch was wired into an oscilloscope. During an experiment, the oscilloscope continuous camera was started and a bead introduced into the tube. When the bead reached the first mark, the switch was depressed and only released when the bead passed the second mark. A 50 Hz

signal was fed into the second channel of the oscilloscope and also recorded on the film to make measurement of the travel time as accurate as possible.

The experiment was repeated with standard solutions in the tube to determine the accuracy of the technique and then repeated with the tube full of statolymph. Six animals were required to provide enough statolymph to fill the tube.

	Experimental Values		Standard Values	
	21.5°C		20°C	25°C
Water	1.00 (±0.036)	1.00 (±0.043)	1.00	1.00
20% Sucrose Solution	1.71 (±0.05)	1.79 (±0.048)	1.945	1.695
Saline	1.06 (±0.036)	1.10 (±0.041)		
Statolymph	1.08 (±0.049)	1.16 (±0.051)		

All values quoted as viscosity relative to water at the same temperature. In each of two experiments 20 values were obtained for each fluid. The mean value for each of those experiments is quoted with the standard deviation.

The technique is obviously not sufficiently accurate to give an absolute measure of the viscosity of the statolymph. However, it does serve to indicate the approximate viscosity compared to, for instance, the saline being used.

Chemical Constitution

The Microanalysis Section of the Research School of Chemistry at A.N.U. analysed the statolymph for inorganic ions. 50 µl of statolymph was diluted in 10 ml of distilled water. At this dilution, the concentration of some of the ions was close to the limits of resolution of the techniques being used. As a known standard and also a measure of the sensitivity of the method, saline was analysed in undiluted form and also at

the same dilution as the statolymph. Undiluted blood was also analysed. 10 ml of fresh blood was added to 10 ml of 10 mM Citric acid. This precipitated a small amount of protein but prevented coagulation of the remaining blood. The precipitate was spun down and the supernatant analysed.

No figures are given for concentration of the chloride ion because the oxygen flash technique used was not sensitive enough to measure the ion in the diluted statolymph. Atomic absorption spectroscopy was used to measure the concentration of the cations.

Table 1

Empirical Results for *Scylla* (ppm)

	Na	K	Ca	Mg
Saline Calculated	12300	520	493	591
Saline Undiluted	11560 (±290)	556 (±9.6)	479 (±6.9)	530 (±14.7)
Saline Diluted	12400 (±145)	669 (±22.2)	326 (±11.6)	573 (±11.5)
Blood	8730 (±355)	409 (±18.4)	389 (±19.1)	459 (±12.9)
Statolymph	12530 (±154)	896 (±49.2)	224 (±16.3)	206 (±22.8)

Three values were obtained for each experimental concentration in separate analyses. The quoted figure is the mean value and the standard deviation is shown in brackets.

Table II

	Na	K	Ca	Mg
Sea Water (Lyman)	10740 ppm	357	411	1305
Sea Water (Sverdrup)	10831	388	410	1288
Carcinus Blood (Webb)	12149	478	529	466
Carcinus Blood (Shaw)	10764	472	700	566
Carcinus Saline (Pantin)	12310	518	497	599
Cancer Serum (Cole)	10576	398	460	525
Callinectes Saline (Sawaya)	9609	450	512	853

(Figures collated from various tables in "The Biology of Marine Animals" by J.A.C. Nicol, Pitman, London 1967.)

Table II has been included here for two reasons. Primarily, it provides some figures for the marine environment and other crabs as a comparison. In addition, it provides an indication of how capricious this kind of analysis is, especially, it seems, when analysing blood. Shaw and Webb differ considerably in their values for Carcinus blood for three out of four ions.

The ionic constitution of the blood is almost certainly affected by many factors, most of which are not controlled for when performing this type of analysis. The age and sex of the animal may be important, together with the type of environment it has been living in. The stage of the moult cycle is known to affect the level of blood Ca (Nicol) and may also affect other ions.

On the basis of the results obtained, Scylla has a lower blood Na level than most marine crustaceans for which figures are available. The statolymph seems to contain significantly higher levels of Na and K than the blood but lower levels of Ca and Mg.

There are significant discrepancies between the

figures for diluted and undiluted saline. Regardless of whether this is due to the sensitivity of the analytical technique or to inaccurate dilution, the results for the statolymph probably err in the same direction, since the same procedure was followed. Thus, the K level in diluted saline is significantly higher than in undiluted saline and the Ca level significantly lower. Therefore, the K level in statolymph is probably lower than 896 ppm and the Ca level higher than 224 ppm. This correction brings the Ca levels of blood and statolymph much closer together but there appears to remain a significant difference between the respective K levels.

One interesting feature of these results is that the statolymph contains higher levels of both Na and K than the blood. In the vertebrate labyrinth, by contrast, the endolymph has a high K and low Na concentration and the perilymph has the reverse (Bosher and Warren 1968).

Dr J.L. Denburg (Department of Neurobiology, A.N.U.) performed a quantitative protein analysis on the statolymph using the Lowry method (1951). A similar determination was done on a blood sample for comparison. The value for blood was 36 mg of protein per ml blood. In two separate experiments, the values for the statolymph were 5 mg/ml and 11 mg/ml.

The reason for the discrepancy in values for statolymph is not known but it is possible that it represents a real difference in the protein levels in the two crabs. This difference could be a reflection of differences between the animals such as age, sex, stage of moult cycle and time in captivity.

The explanation for the presence of protein in the statolymph may possibly be related to the pores in the cuticle, mentioned earlier. This will be discussed in the next section.

Statolith Hairs

Near the bottom of the vertical canal, on the vertical wall of the keel, just below the sensory cushion, there is a yellow-coloured mass, approximately 350 μm in diameter, projecting from the surface into the fluid; this is the statolith (Fig. 7a). Partly because of the colour, and partly by extrapolation from other decapods, it is thought to consist of sand grains bound together in some way, possibly in a mucoprotein matrix.

Around the edges of the statolith are two incomplete rings of hairs, both rings being broken at the dorsal extremity so that they resemble concentric horseshoes (Fig. 7b). The hairs in both rings are extensively feathered at the tip (Fig. 7c). The main stem of the inner hairs is about 4 μm wide and 40 μm long, but the feathering at the tip probably adds 25-30 μm to the length. The outer hairs are slightly larger, perhaps 5 μm longer. The side branches that constitute the feathering on both types of hair measure approximately 0.1 μm in diameter.

The tips of all the hairs, inner and outer, are bent over towards the centre of the ring. The statolith appears to sit over the inner hairs and its edges are just in contact with the tips of the outer hairs. In the scanning electron microscope, it can be seen that the feathering of the inner hairs is intimately enmeshed in the substance of the statolith.

The statolith is the main position monitor in the statocyst. The hairs have a tonic output, which is set by the shear force acting on each hair as a result of the statolith mass. Any change in magnitude or direction of linear acceleration, such as that of gravity, will cause a slight shifting of the mass, with resultant change in the shear

Figure 7

Scanning electron micrographs of the statolith region.

a. 45X *The statolith in position on the vertical wall of the keel.*

b. 65X *Statolith removed to show the two concentric rings of hairs. Unfortunately the rings are not intact in this specimen due to damage during preparation.*

c. 1500X *Hairs are extensively feathered at the tip.*

d. 300X *Holes in the cuticle within the ring of hairs compared to "pores" in the cuticle near the statolith area.*

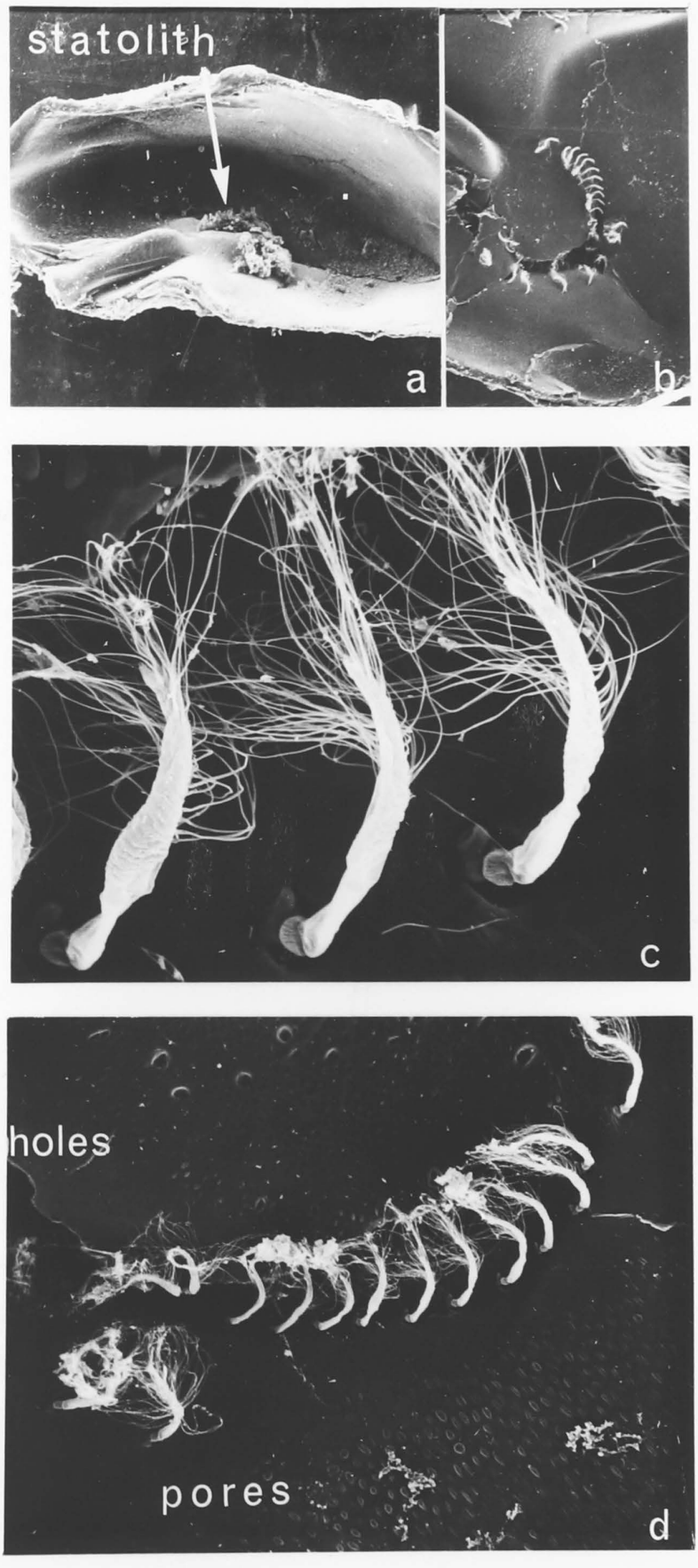


FIG. 7

forces acting on some or all of the hairs. Details of the innervation of the statolith hairs are unknown for Scylla except that Sandeman & Okajima (1973) reported a ring of very large cell bodies associated with the hairs of the outer ring. No experiments were performed on these hairs but some information was acquired incidentally. This will be presented in Chapter 6.

In the centre of the ring of hairs, beneath the statolith, there are holes in the cuticle (Fig. 7d). There are about 20 large holes (5 μm) and a very large number of much smaller ones (1 μm) and neither shows any resemblance to the pores mentioned earlier in the chapter (P.18). These pores can also be seen in Fig. 7d and in Fig. 8.

Prentiss (1901) reported the existence of pores in the statocyst cuticle in most of the decapods he studied and he was able to trace ducts from the pores to small gland cells embedded in the tissue surrounding the statocyst. He suggested that the glands might produce a substance which was required in the statocyst to "stick" the statoliths to the underlying sensory hairs.

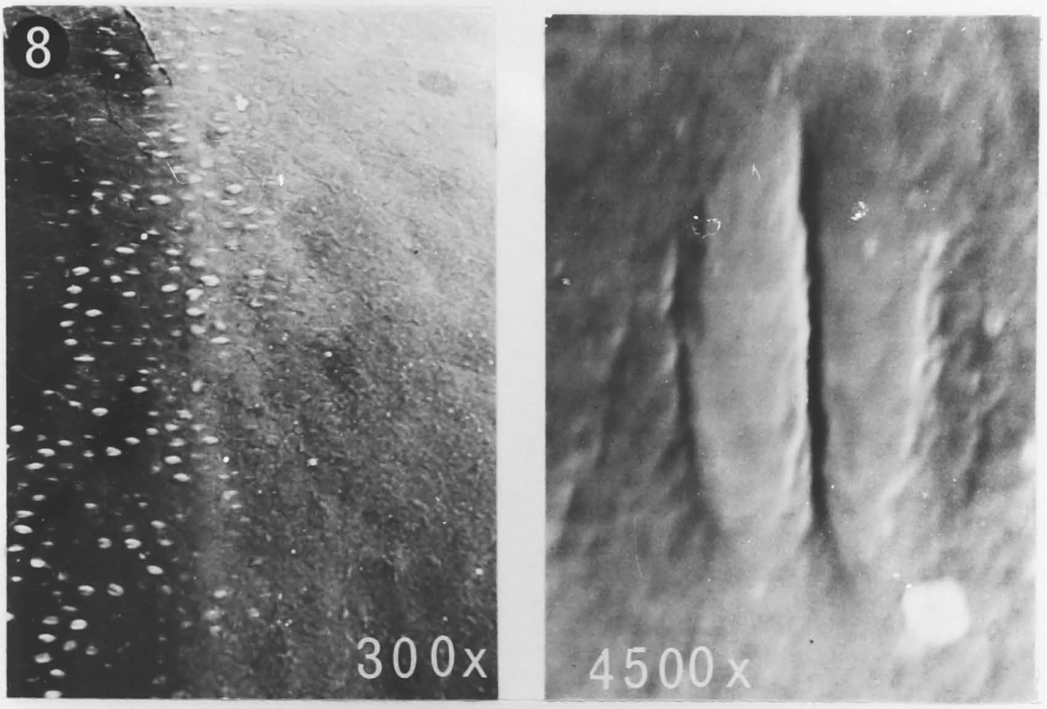
Lang and Yonge (1935) investigated these tegumentary glands in the lobster statocyst. They found that the glands opened into the statocyst via a duct with a characteristic funnel-shaped ending. They also found that the glands secreted the cuticular layer on the inside of the statocyst. Further analysis showed that the substance used to bind the statolith to the sensory hairs had many properties in common with the cuticle. They concluded that the tegumentary glands probably also secreted the sticky substance.

Figure 8

Scanning electron micrographs of pores found on the sensory cushion near the statolith.

Figure 9

10 µm wax section of presumed gland cells in the tissue around the sensory cushion. Stained with Mallory's Triple Stain.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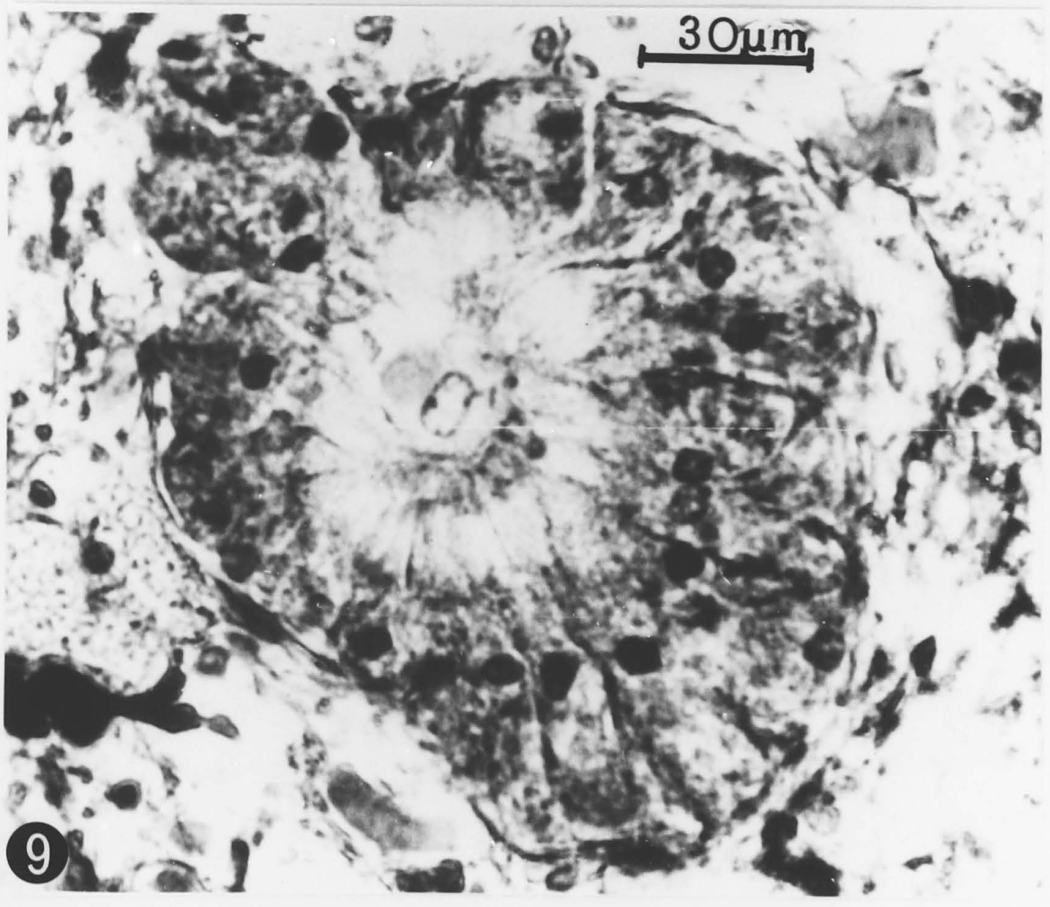
300x
4500x


30um
9

In Scylla, cellular structures bearing a very close resemblance to the gland cells of Prentiss have been found in the connective tissue around the statocyst, especially around the sensory cushion (Fig. 9). No ducts have been found linking these glands to the statocyst cuticle but it seems likely that they are the same glands as those described by Prentiss.

Although no analysis comparable to that of Lang and Yonge has been carried out on Scylla, the larger pores beneath the statolith do have a funnel-shape and are strategically located to secrete statolith-related material. It is even possible that, as in some other ~~decapods~~ crustaceans (Mysis, Bethe 1895) the whole statolith is secreted by the animal and not taken in from outside.

The protein found in the statolymph may be secreted by one or both types of pores. For instance, if the pores beneath the statolith do secrete a sticky substance, perhaps it is a mucoprotein.

Another possibility is that the thread hairs are enveloped in a cupula, proteinaceous or otherwise, similar to that of the vertebrate semi-circular canals. No evidence of such a cupula has been found, but it took a long time to establish conclusively its existence in vertebrate systems, because of its fragile and transparent nature. The pores are grouped into two patches at either end of the sensory cushion adjacent to the two areas where the thread hairs are most densely grouped.

There are many other possible explanations for the presence of protein in the statolymph and pores in the cuticle. For instance, it seems likely that the viscosity of the statolymph is a critical factor in the dynamic behaviour of the

system. Perhaps the variability in canal dimensions in animals of different sizes is offset by compensatory changes in the viscosity of the statolymph. This could be achieved if the animal is able to control the level of protein in its statolymph by secretion and/or absorption. Viscosity is also very temperature dependent, so perhaps the animal can even alter the protein level to maintain a uniform viscosity of the statolymph at different temperatures.

Free Hook Hairs

The free hook hairs are like the statolith hairs, except that they are larger. They are extensively feathered at the tip and measure 5-6 μm in diameter and 60 μm in length without the feathering. They are bent over at the tip, but there does not appear to be any pattern to the direction of bend of the tips or indeed to the distribution of the hairs (Fig. 10). They occur in a patch in the medial arm of the vertical canal, running for about 1.5 mm from the top edge of the keel almost up to the common canal (Fig. 11). The patch is wider at the top (200 μm) than at the bottom (100 μm).

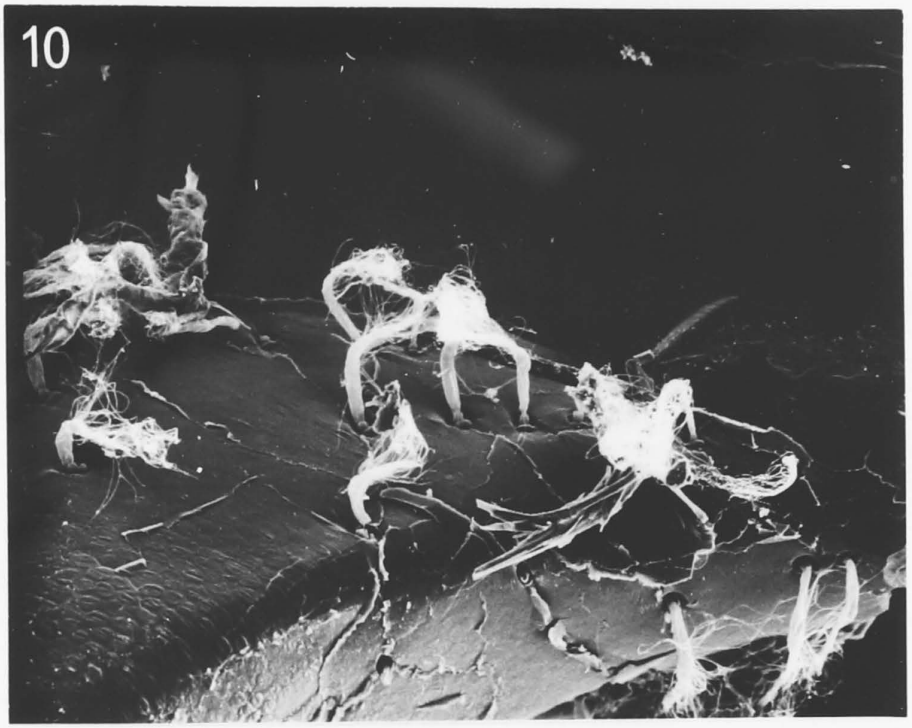
The function of the free hook hairs is unknown. Sandeman and Okajima (1972) have shown that they are intermediate in sensitivity between statolith hairs and thread hairs to a jet of saline directed at them and Fraser (personal communication) has shown a response to high frequency (20-100 Hz) sinusoidal oscillation. Taken together, these two pieces of information have elicited the suggestion that the free hook hairs are high frequency dynamic receptors which take over when the much more sensitive thread hairs reach the upper limits of their response range. This was also hinted at by

Figure 10

Scanning electron micrograph of a small area of the patch of free hook hairs. The extensive feathering of the hairs is obvious and it can also be seen that the distribution of the hairs is apparently random.

Figure 11

Statocyst floating in saline after staining with methylene blue. The patch of free hook hairs and the concentric horseshoes of the statolith hairs can be clearly seen through the almost transparent cuticle of the medial arm of the vertical canal.



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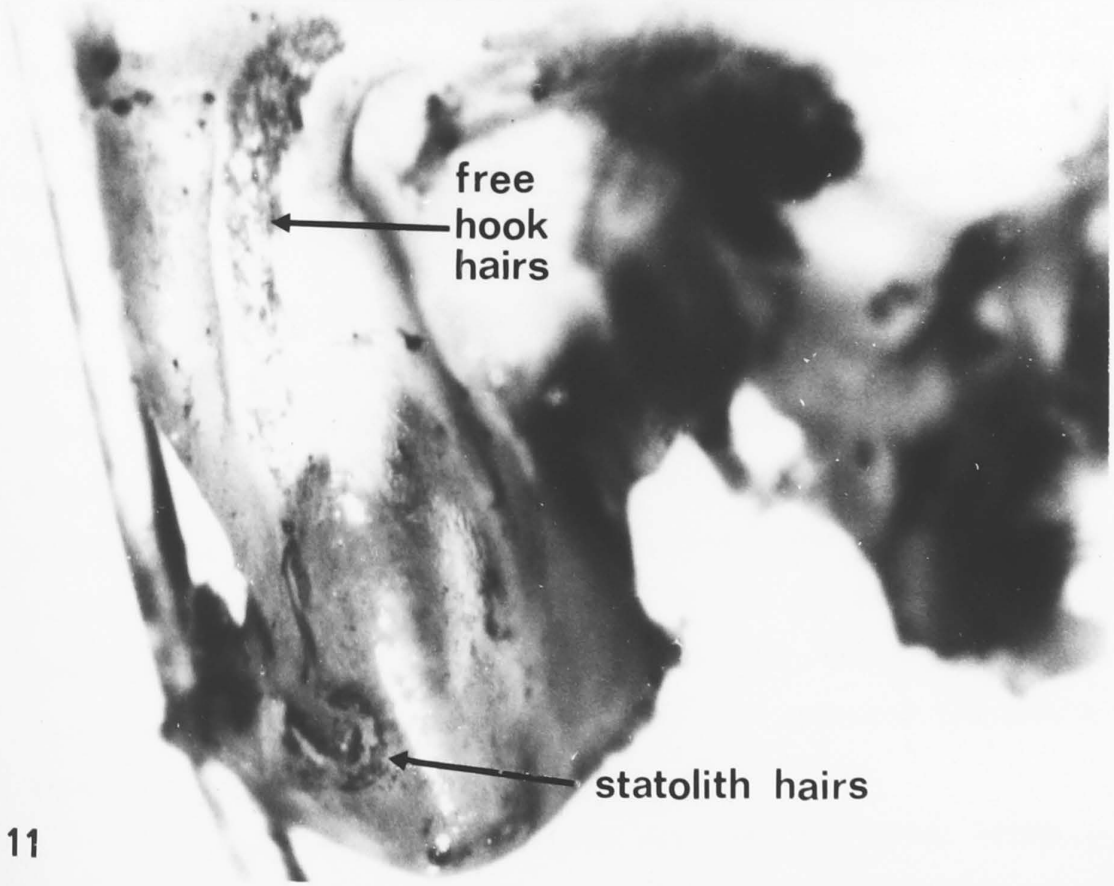


11

0·5mm

Cohen and Dijkgraaf (1961). No work, however, has been done on the free hook hairs. All that is known of their innervation is the sub-bundles of the antennulary nerve that supply them (Sandeman and Okajima 1972).

Group Hairs

In the horizontal canal, close to the lateral junction of the two canals is an area of much larger, thicker hairs, the group hairs (Fig. 12). These measure 15 μm in diameter and 400-500 μm in length. Scanning electron micrographs reveal that they are not feathered and, in this respect, they resemble those of Carcinus (Prentiss 1901).

No nerves have been traced to the group hairs in any decapod, with the result that their function remains obscure. In this study, one set of hairs was embedded in resin and sectioned. Longitudinal sections show that the hair shaft is not hollow but is quite densely packed with tissue of a heterogeneous nature (Fig. 13). However, no neurons were found associated with the hair.

It has been suggested (Dijkgraaf 1961) that the group hairs have a function in controlling fluid flow in the statocyst. As the statocysts are arranged in the animal, the group hairs lie at the most distant point from the midline, in an area of the statocyst which possibly experiences the strongest fluid flows during yawing movements of the animal. Perhaps they damp the fluid flow in the horizontal canal, preventing over-stimulation of the upper thread hairs and reducing the fluid flow into the vertical canal.

Prentiss (1901) pointed out that the group hairs resemble some of the tactile hairs found on the outside of the

Figure 12

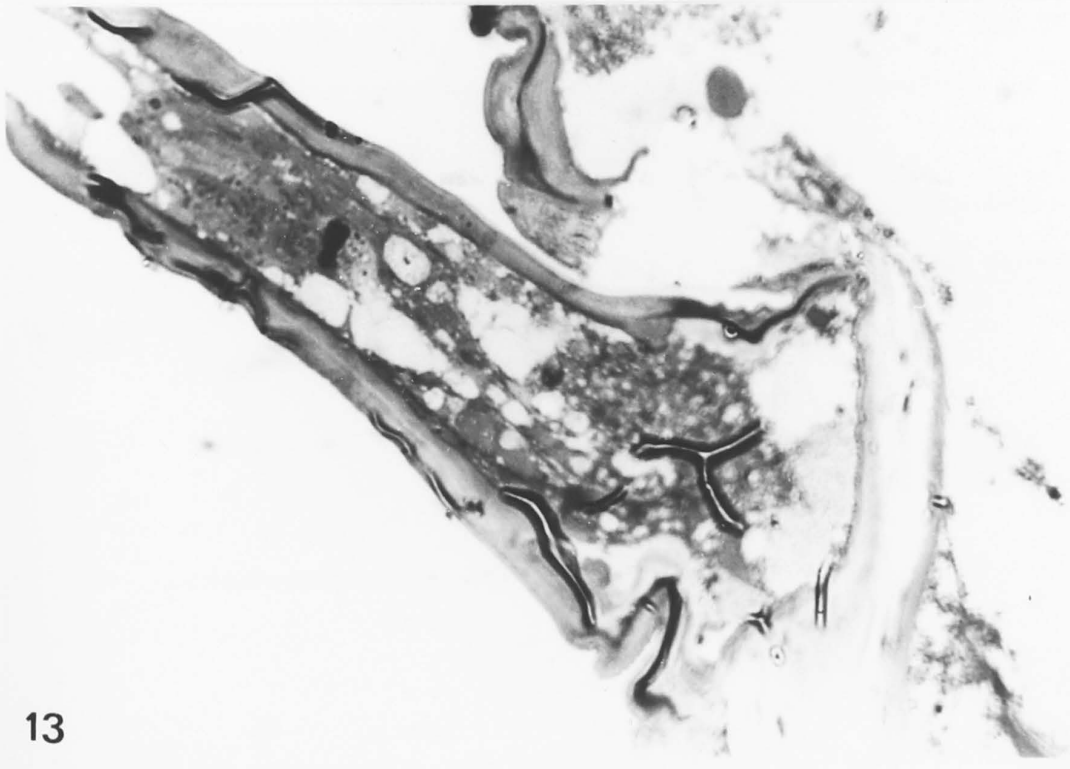
Scanning electron micrographs of the group hairs to show their location and arrangement and the smooth, unfeathered shafts.

Figure 13

1 μm section of the base of a group hair stained with toluidine blue to show the heterogeneous tissue inside the shaft.

12

80X 700X



13

10µm

basal segment of the antennule and suggested that they may have been included in the statocyst "accidentally" when the invagination of the segment wall occurred. This resemblance between the two hair types is also true for Scylla. However, even if the hairs did have rather inauspicious origins, they may now fulfil an important role in the functioning of the statocyst.

Thread Hairs

The thread hairs occur in a roughly vertical, single line along the centre of the sensory cushion. Each hair is extensively feathered and measures only about 2 μm wide but up to 500 μm long, hence the name "thread hairs". The hairs project out into the statolymph, mainly into the common canal at the top of the cushion and into the keel at the bottom of the cushion. They are very sensitive to any movement of the fluid in the canals and are the principal dynamic receptors in the statocyst.

The remainder of this thesis will describe the detailed structure, innervation and behaviour of the thread hairs.

CHAPTER FOUR

Thread Hairs - Anatomy and Innervation

Introduction

Hensen (1863) first described and named the thread hairs. In Carcinus he reported them to be very long and thin and feathered at the tip. He also described the flexible spherical membrane at the base which appeared to give the hairs great freedom of movement. In addition, he described a structure which he called a "lingula" that projected from the inner wall of the hair shaft into the spherical membrane and was the point of attachment for the nerve.

Prentiss (1901) added that the thread hairs are feathered along their length in the Megalops larva of Carcinus and confirmed Hensen's impression that there was a single nerve to each hair. No more information is available on the innervation and structure of the thread hairs. However, Schöne and Steinbrecht (1968) have described the ultrastructural innervation of statolith hairs in the crayfish and the reports referred to above suggest that the structure and innervation of statolith and thread hairs is similar.

This chapter describes the structure, arrangement and innervation of the thread hairs of Scylla serrata.

Arrangement of the Hairs

In longitudinal section, the profile of the sensory cushion is approximately one half of an ellipse sectioned along the major axis (Fig. 1). Consequently, as the hairs project from the cuticle approximately perpendicular to the surface, the hairs at opposite ends of the cushion will be at an angle of nearly 180^{o} to each other, whilst those at the centre of the cushion will be perpendicular to those at either end.

The hairs run in a single line approximately down the centre of the cushion (Fig. 3). This line is not straight but

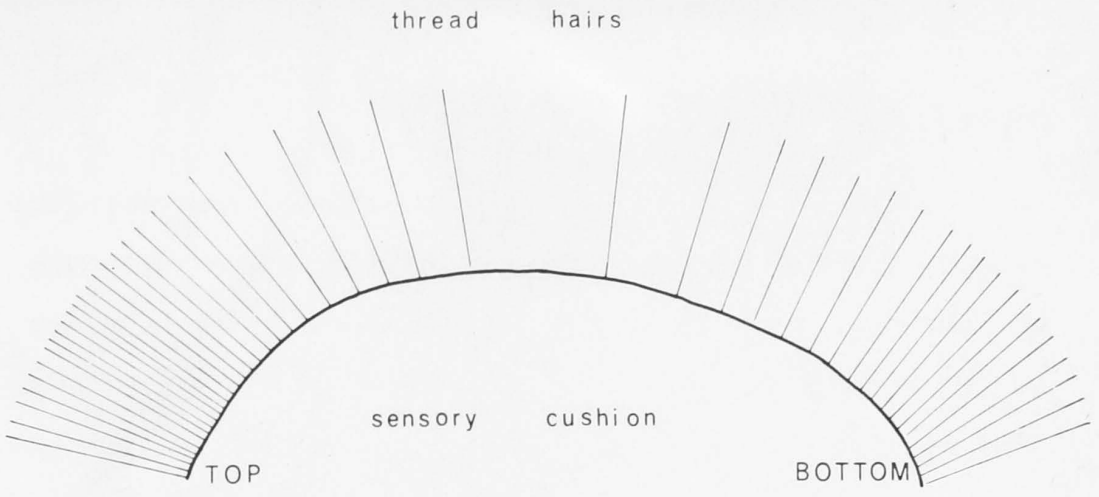


FIG. 1 Schematic profile of sensory cushion. (not to scale)

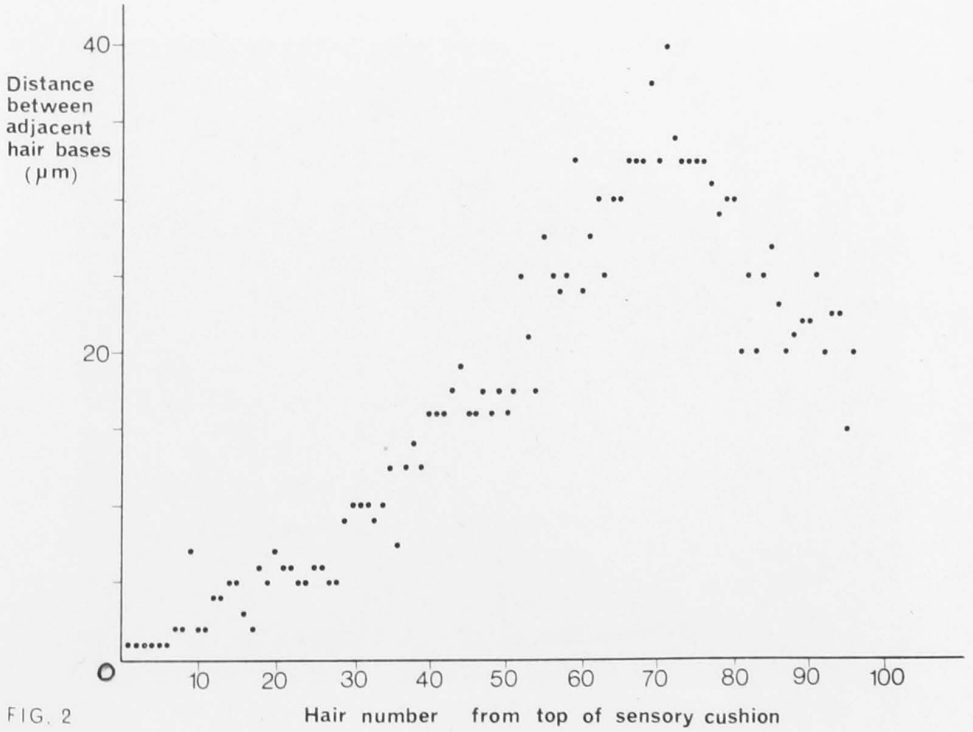
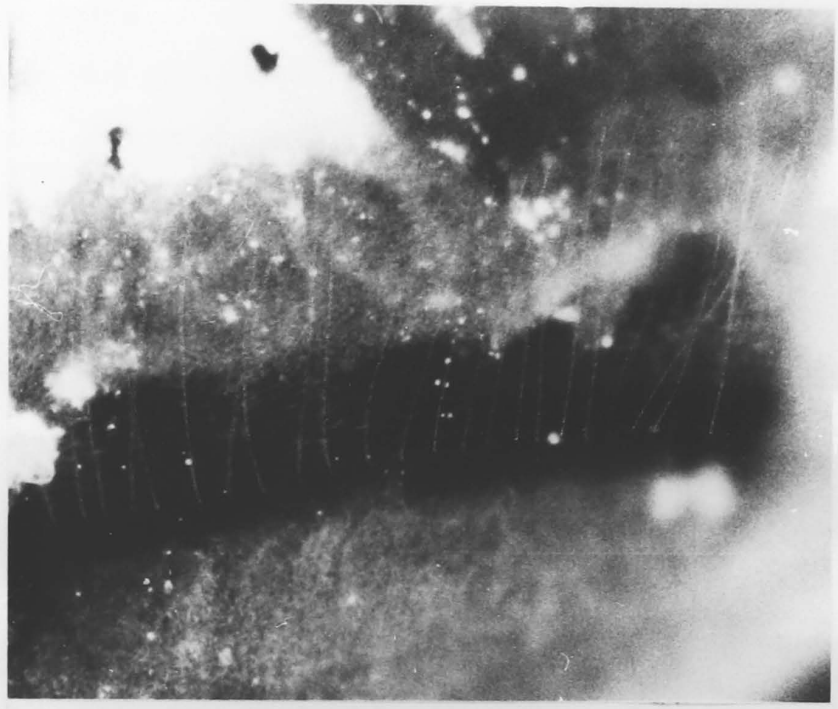


FIG. 2

Figure 3

Line of thread hairs on the sensory cushion. Upper photograph shows the thread hairs floating upright in saline. Lower photograph is a scanning electron micrograph. The hairs, unfortunately, do not retain their upright stance during the processing, although critical point drying does prevent them from collapsing completely against the sensory cushion.



65 X

FIG. 3

has a very distinctive form and the form in one statocyst is a mirror image of that in the contralateral statocyst. In the central third of the sensory cushion, the line of hairs is straight and runs parallel to the sides of the cushion. However, the upper and lower thirds of the line are gently curved in opposite directions so that the whole line has an elongated S-shape. If the sensory cushion is viewed from the concave surface, the normal S occurs in the right statocyst whilst the mirror image occurs in the left. Put another way, in both statocysts, the line of upper hairs curves towards the midline of the animal, whilst the line of lower ones curves away. This can be seen in figure 6, chapter 3. The statocyst is, as described earlier, tilted out of the vertical plane in its normal position in the animal. It is possible that the position of the statocyst is such that the upper and lower hairs coincide exactly with the vertical plane. The direction of tilt of the statocyst is appropriate for this to occur.

An important point about the thread hairs is that they are not distributed evenly along the cushion. They are densely packed at the bottom of the cushion, even more densely packed at the top, and much more widely spaced over the central region. A plot of the distribution of hairs along the length of the cushion can be seen in figure 2.

A further point concerns the hairs at the centre of the cushion and draws together three earlier observations. The sensory cushion comes within 50 μm of the opposite wall at the centre of the vertical canal and yet the thread hairs measure up to 500 μm in length. There are not many thread hairs in the centre of the cushion but it was thought that these may be forced out of their perpendicular position by the invaginated

opposite wall. However, a transverse section of the vertical canal (Fig. 5, chapter three) reveals that the invaginated wall opposite the sensory cushion actually consists of two invaginations with a trough between them, as seen from inside the canal. The sensory cushion comes within 50 μm of one of the invaginations but the line of thread hairs lies in a position on the sensory cushion opposite the trough. This allows the thread hairs to project normally from the sensory cushion.

Single Hair

An individual hair is feathered along its entire length, but the feathering appears to be longer and more dense at the tip of the hair (Fig. 4). Dijkgraaf (1956) reported that the thread hairs of Carcinus are feathered only at the tip but that those of Maja verrucosa are feathered along the entire length. Scylla thus more closely resembles Maja in this respect, although it is possible that, with the aid of the scanning electron microscope, fine feathering may subsequently be found on the shafts of the thread hairs in Carcinus.

The length of the lateral filaments on the thread hairs is greater than half the distance separating the hairs so that the feathering of adjacent hairs could overlap and interlock. The interlaced hairs would present an effective curtain to the fluid in the canal.

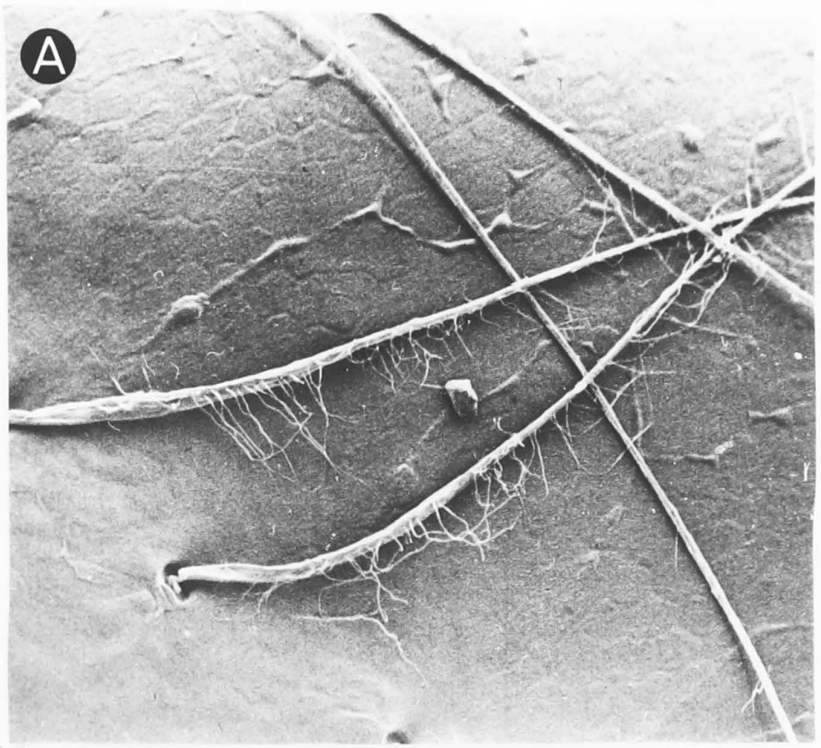
Occasionally, in whole mounts or in the scanning electron microscope, a discontinuity is seen on the shaft of the thread hair (Fig. 5). It consists of a slight symmetrical dilation of the shaft with a horizontal line across the shaft at the widest point and is probably just a fracture caused by bending the hair shaft. However, the possibility exists that it is a permanent feature of the shaft, perhaps a flexion point.

Figure 4

Scanning electron micrographs of the thread hairs.

A. *Feathering on the hair shaft near the base of the hair.*

B. *Longer and more abundant feathering at the tip of the hair.*



650X

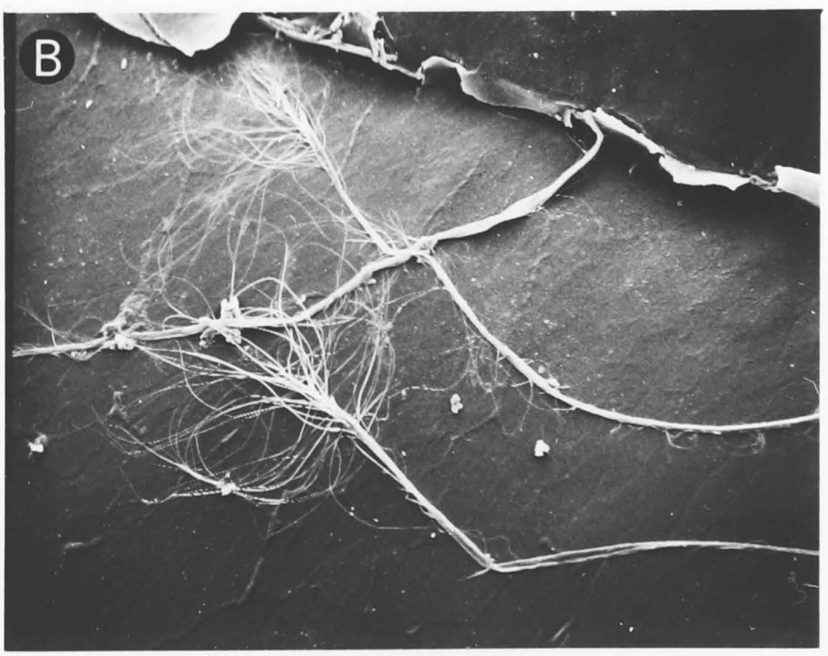


FIG. 4

Figure 5

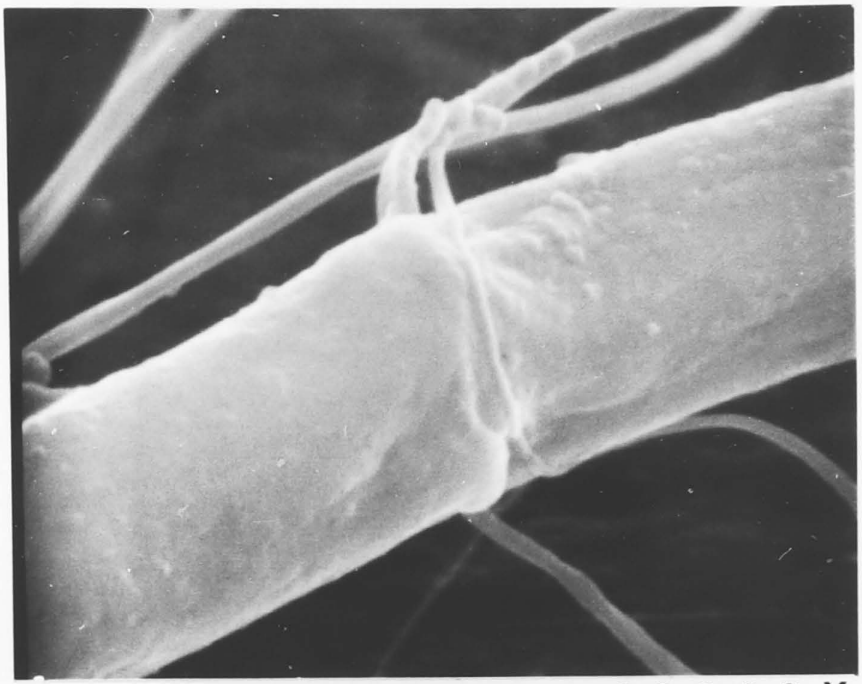
High magnification scanning electron micrograph of the shaft of the thread hair showing a curious discontinuity which may possibly be a flexion point along the shaft.

Figure 6

a. *A line of hair bases, as seen from outside the sensory cushion, to show the asymmetry of the bases.*

b. *Scanning electron micrograph of a series of hair bases from which the shafts have broken away. The ridge and trough are clearly visible and the outline of the ribbed structure can be seen arising from the trough.*

5



20,000 X

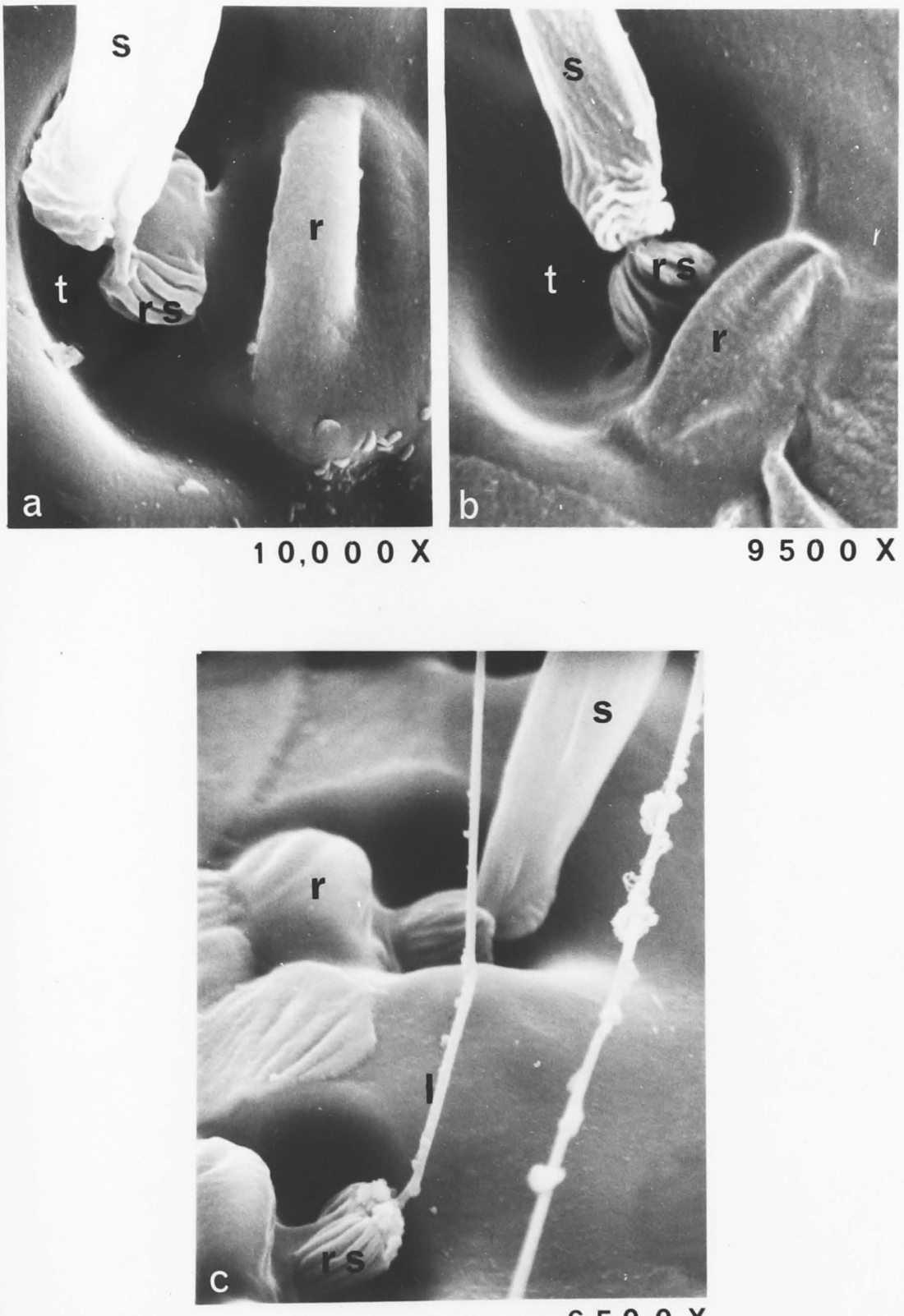
6 a

b

800 X

Figure 7

Three scanning electron micrographs show the detail of the thread hair base from different angles. The ribbed structure (r.s.) arises from the trough (t) below the ridge (r) and supports the shaft of the hair (s). In (c) two adjacent bases are shown and the base in the foreground demonstrates a phenomenon seen occasionally. A very slender rod (1) is all that remains of the thread hair shaft. This may possibly correspond to the lingula (a cuticular spine of the hair shaft) reported by Schöne and Steinbrecht (1968) in the statolith hairs of the crayfish.



10,000 X

9500 X

6500 X

FIG. 7

Figure 8

Electron micrograph of thread hair base showing cup-shaped depression or trough (T), cuticular ridge (R) and sclerotised spherical membrane (RS) arising from the trough. The hair shaft has broken off at the open end of the spherical membrane. Arrowed is the tubular process which derives from the sensory nerve and inserts at the base of the hair shaft (slightly oblique longitudinal section).

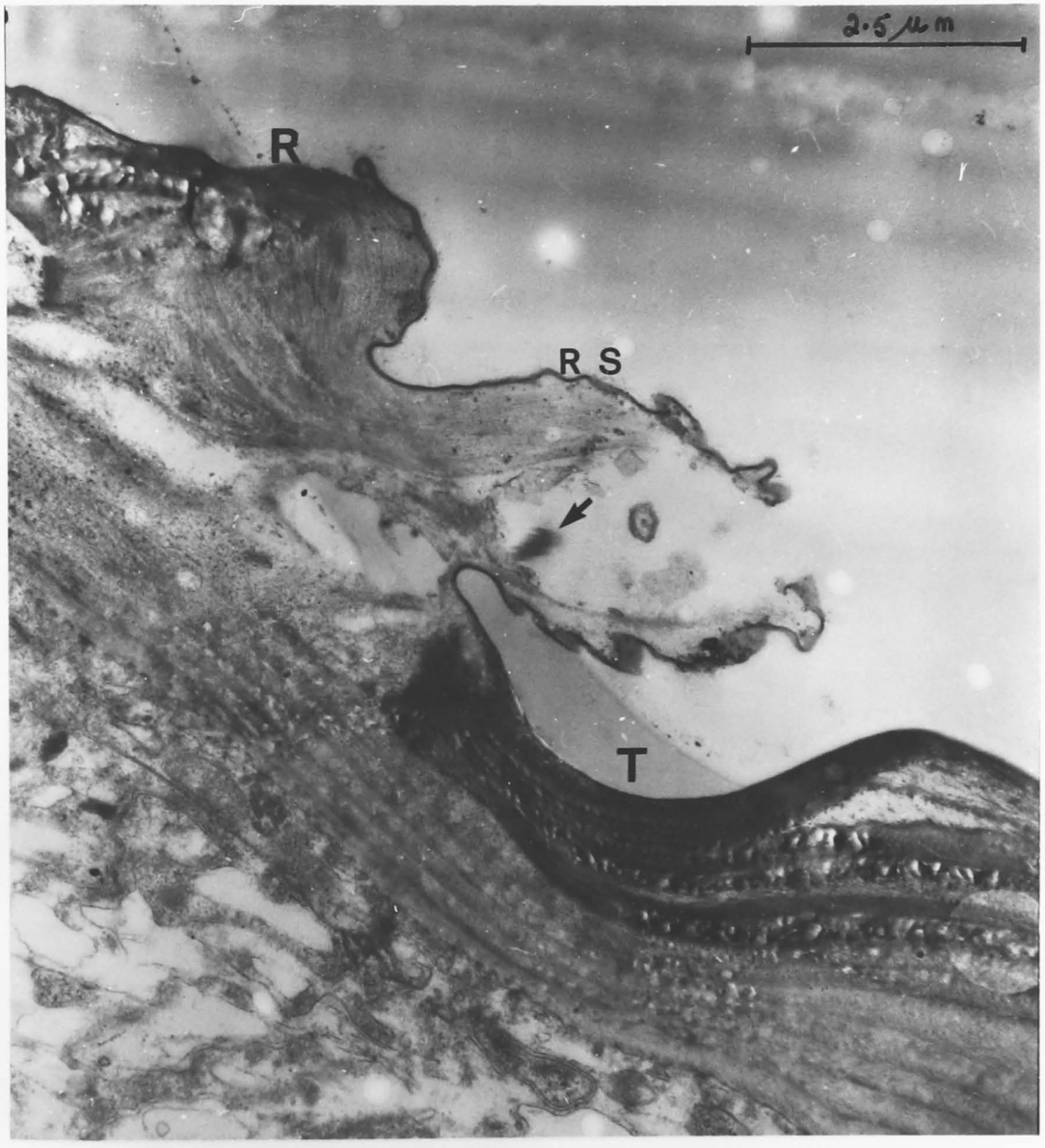


FIG. 8

Hair Base

The hair base is asymmetrical about a line drawn through all the bases (Fig. 6). Each base is circular or slightly elliptical and measures 10-12 μm in diameter. It consists of a cup-shaped depression in the cuticle with a cuticular ridge on one lip of the depression. The ridge is always on the cell-body side of the base. Arising from the depression below the ridge is a roughly spherical structure with prominently ribbed walls (Fig. 7). This corresponds to the "spherical membrane" of Hensen (1863) and the "sclerotised cask-shaped hair base" of Schöne and Steinbrecht (1968) and it can be seen in the light microscope, the scanning electron microscope (Fig. 7) and the transmission electron microscope (Fig. 8). The shaft of the hair arises from the upper surface of this sclerotised sphere and appears to articulate with it via a narrow neck.

In the scanning electron microscope the spherical base can occasionally be seen to support a smooth, slender rod, very much narrower than the usual hair shaft (Fig. 7C). Schöne and Steinbrecht described the sensory process of the statolith hair as inserting on a cuticular spine of the hair, called the "lingula". The structure seen in figure 7C could well correspond to such a structure in the thread hair of Scylla, the remainder of the shaft having become detached during processing for microscopy.

Innervation

Sandeman and Okajima (1972) traced the various bundles of the antennulary nerve to their peripheral destinations. There are three main bundles and they found that sub-bundle A of bundle II carried only thread hair axons. Subsequent work by

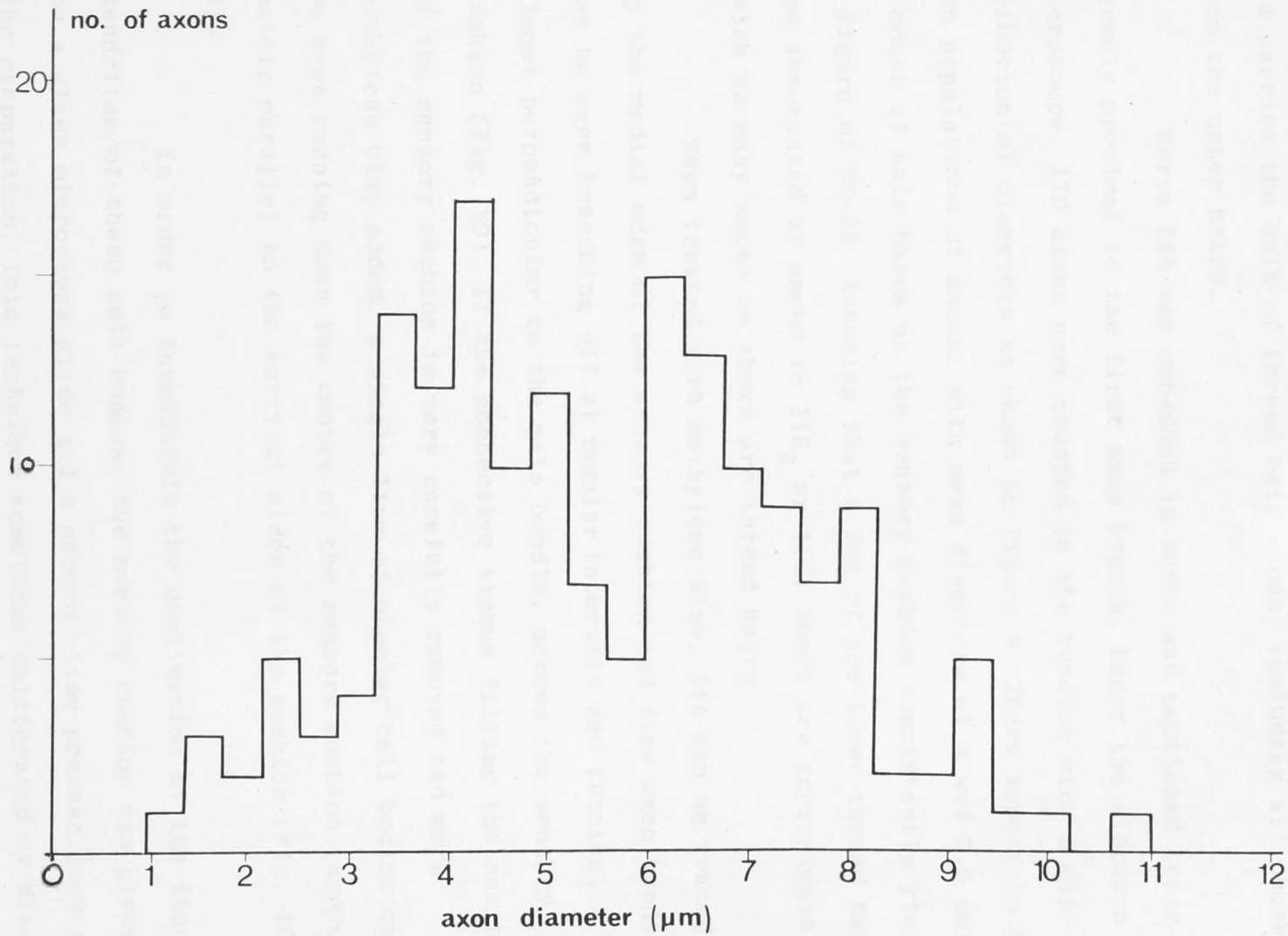


FIG. **9**

Silvey (in press) has shown that bundles IIC and IIB_2 occasionally contain axons from the lower thread hairs but that IIA carries the bulk of thread hair axons, including all those from the upper hairs.

Nerve IIA was embedded in resin and sectioned transversely proximal to the first axon branch. Under the electron microscope, 170 axons were counted in the bundles with a distribution of diameters as shown in figure 9. There appear to be two populations of axons, with mean diameters of 4 and 6.5 μm. A count of hair bases on the sensory cushion consistently yields a figure of 90-95. Assuming that a few of the lower thread hairs are innervated by axons in IIB_2 or IIC, there are approximately twice as many axons as there are thread hairs.

When treated with methylene blue, IIA can be traced up the medial edge of the sensory cushion and fine axon bundles can be seen branching off at regular intervals and running, almost perpendicular to the main bundle, across the sensory cushion (Fig. 10). If the connective tissue filling the cavity of the sensory cushion is very carefully removed and more methylene blue added, a single line of bipolar cell bodies can be seen running down the centre of the sensory cushion, approximately parallel to the vertical sides of the cushion (Fig. 10A, B).

In order to investigate the destination of the fine dendrites of these cell bodies, the sensory cushion was placed on a glass microscope slide and a second slide pressed down on the preparation. This technique sometimes obliterated or distorted the relevant areas but gave very satisfactory results in most cases. The preparation must be drawn or photographed quickly before the dye fades.

Figure **10**

Methylene blue preparations of a sensory cushion to show the cell bodies of the thread hair nerves.

A. *The location of the cell bodies on the sensory cushion (SC). The line of thread hairs (h) can also be seen.*

B. *The line of cell bodies at higher magnification. The parallel axons (ax) and converging dendrites (d) are clearly visible.*

C. *The dendrites converge and meet in an oval body (ob).*

D. *Single strands join the oval bodies to the hair bases (hb).*

E. *High magnification of oval bodies.*

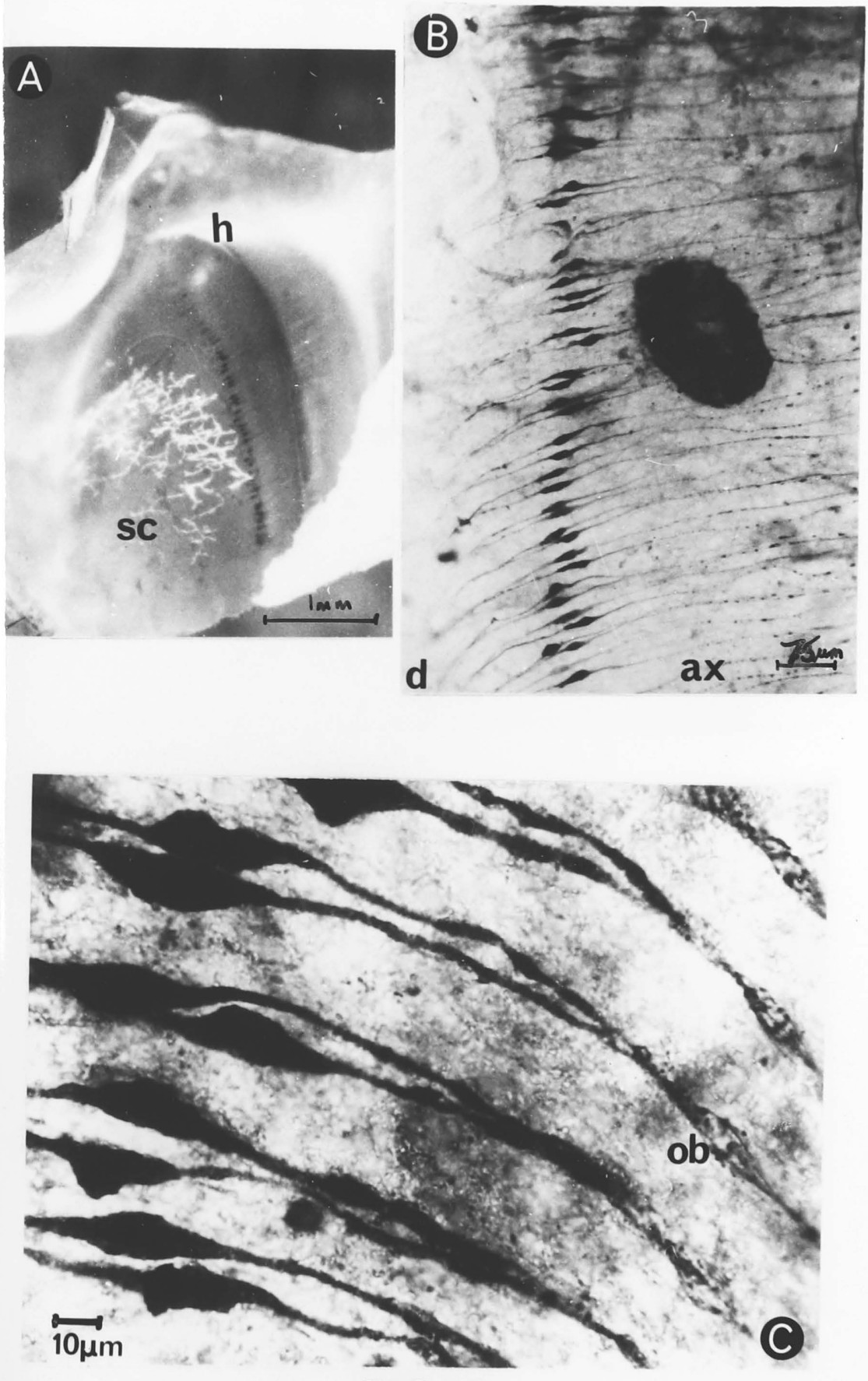
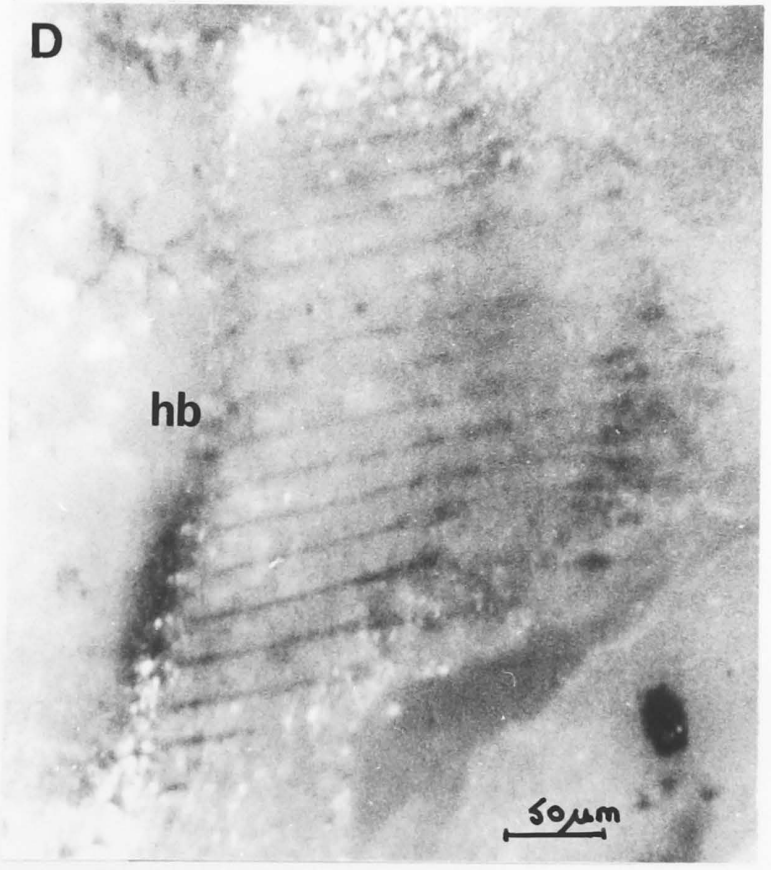


FIG.10



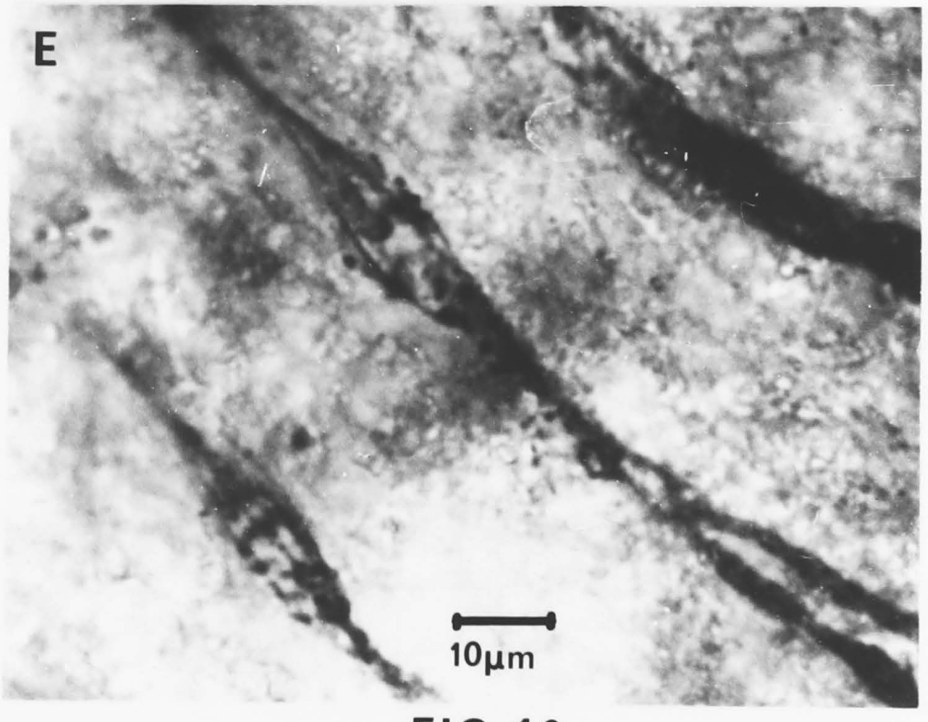


FIG. 10

The cell bodies measure approximately 10 µm in width and 25-30 µm in length and they are arranged in pairs (Fig. 10B, C). The separation between paired cell bodies is consistently less than that between unpaired cell bodies, at the centre or the end of the sensory cushion. If the dendrites are traced from the cell bodies across the sensory cushion, the two dendrites from each pair of cell bodies can be seen to converge and meet in a structure which I ~~have~~ initially called an "oval body" (Fig. 10C, E). From the distal end of the oval body, there emerges what appears to be a single strand of material. This can be traced further across the cushion and ends at the base of a thread hair (Fig. 10D). The distance between the cell bodies and the hair base is approximately 300 µm and the oval bodies occur about halfway between the two.

Each thread hair is thus supplied by two dendrites although only one strand appears to actually enter the hair base. The ultrastructure of the transition from two dendrites to one strand and of the attachment of this strand to the hair base was investigated with the electron microscope.

Ultrastructure

The hair base and the structure connecting it to the oval body were investigated with the aid of the electron microscope (Fig. 11, ~~12~~ 13). Many similarities were found to mechanoreceptor systems described in other statocysts (Schöne and Steinbrecht, 1968), in chordotonal organs of crustaceans (Whitear, 1962; Mill and Lowe, 1973), and in insects (Thurm, 1964, 1965; Young, 1973). However, just as the structures described in these reports all differ in some aspects, so the thread hair receptor system of Scylla shows some individuality.

Figure 11

Electron micrographs of transverse sections of the scolopidial transduction apparatus of the thread hairs. Calibration is 0.5 μm in all cases.

A. *The dendrites (d) are close together in a tubular structure, the enclosed space of the scolopidium. Each contains an electron-dense ciliary root (r). The tube is enclosed by* enveloping ~~sheath~~ *cells (sh), the inner one of which contains the electron-dense scolopale material (s). There appear to be desmosomal contacts (clear arrows) between the dendrites and the scolopale cell.*

B. *The base of a cilium in each dendrite just distal to the basal body (CB).*

C. *The cilia (C) lie free in the enclosed space of the scolopidium. The scolopale material (S) forms an almost complete ring.*

D. *One cilium breaks down into a loosely packed array of microtubules (CT). Both cilia break down this way but one usually does so before the other.*

E. *The microtubules become densely-packed (CP) in both cilia perhaps with loss of some.*

F. *The two rods of densely-packed tubules (CP) become surrounded by a dense material (arrow) and the enclosed space also appears to fill with a less dense material. The scolopale material has disappeared from the* enveloping ~~enclosing~~ *cells by this stage.*

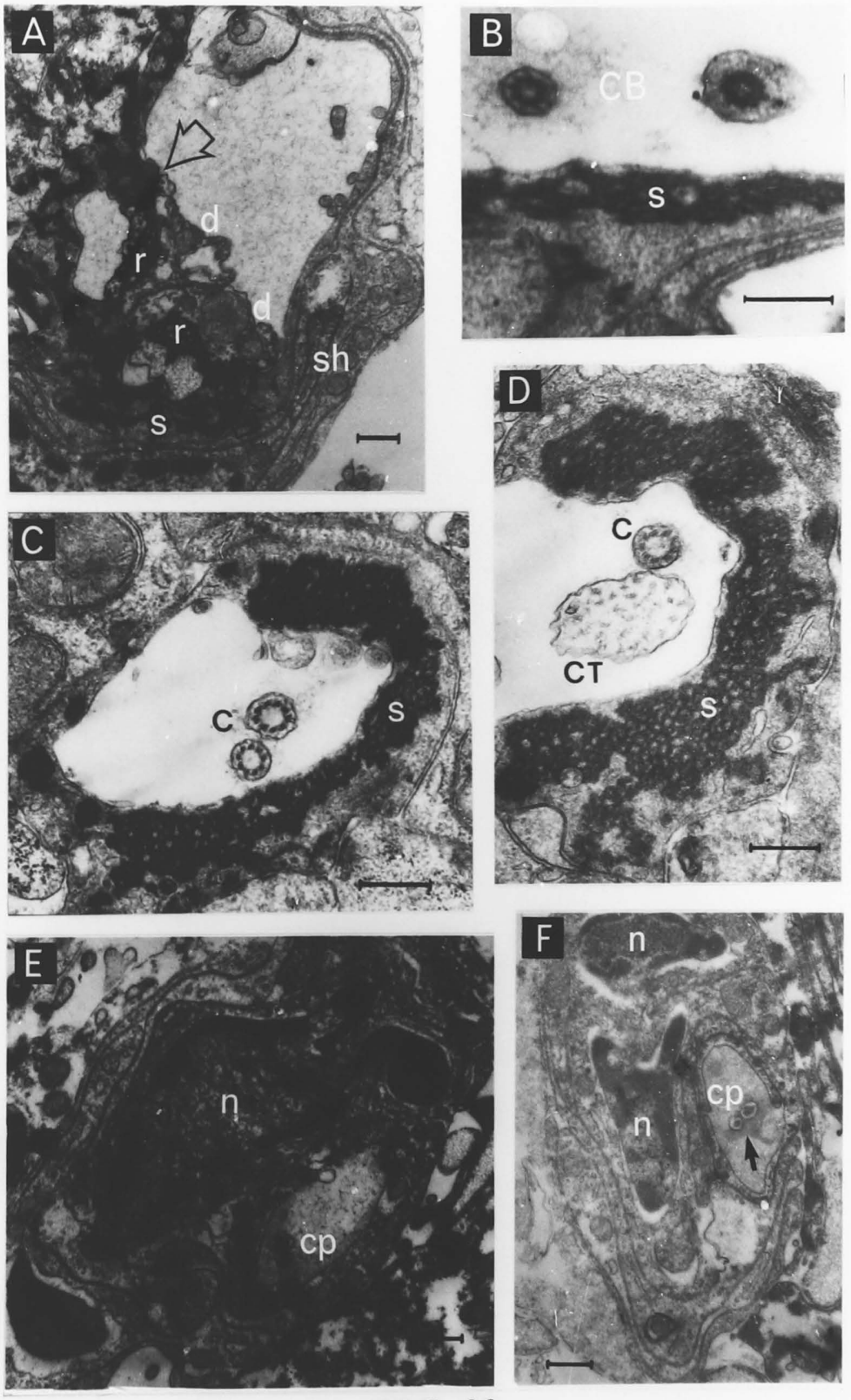


FIG. 11

G. *Only the dense material (arrow) remains in a less dense matrix (m). The cell around the matrix contains longitudinally oriented microtubules.*

H. *The dense material (arrow), presumed to be the chorda, appears the same immediately under the hair base.*

J. *Immediately before the passage through the cuticle to the hair, the* ~~*sheath*~~ enveloping *cells disappear. Only the microtubules remain around the chorda (arrow).*

K. *The chorda (arrow) in the spherical membrane at the base of the hair.*

n: nucleus of sheath cell.

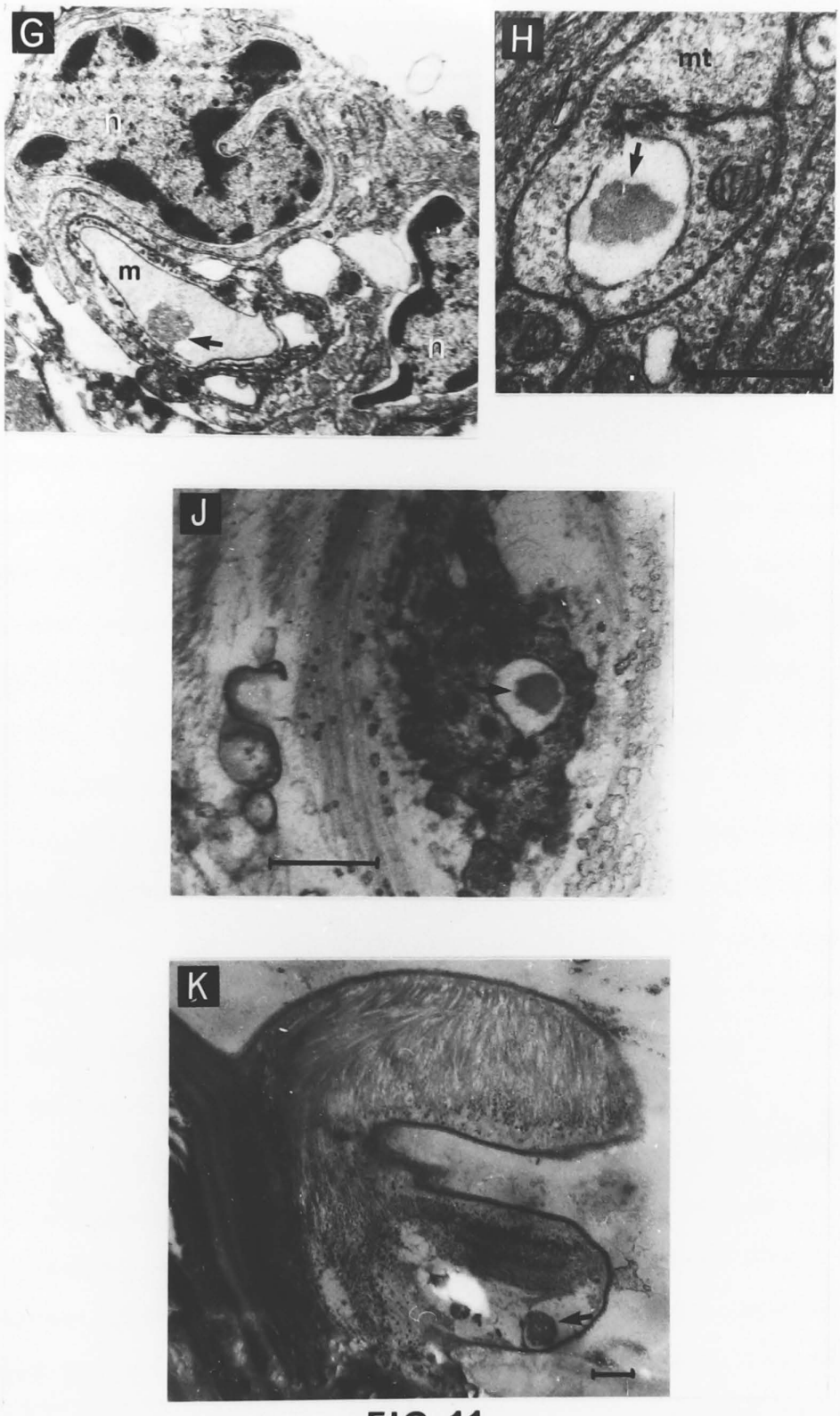


FIG.11

The "oval body" described in the previous section has the basic structure of a scolopidium, a common feature of the mechanoreceptors referred to above. Figure ~~13~~ 12 is a schematic diagram of this structure in Scylla. In transverse section, the scolopidium resembles a hollow tube, approximately 4 μm in diameter, proximally. Around this are several layers of ~~sheath~~ enveloping cells, the inner ~~layer~~ cell containing the characteristic electron-dense scolopale material. At the proximal end this does not form a complete circle around the tube. Within the tube, occupying about one quarter of the enclosed space, the two dendrites can be seen close together (Fig. 11A). Desmosomes can usually be seen connecting the dendrites to the ~~innermost sheath~~ scolopale cell. Each dendrite contains an irregularly shaped, electron dense body measuring approximately 1 μm. This is thought to be the root of the cilium that emerges from each dendrite slightly more distally in the scolopidium (Fig. 11B, C). A basal body can be seen within the dendrite (Fig. 11B) and the cilia then emerge and stand free in the enclosed space of the scolopidium (Fig. 11C). One cilium arises slightly more distally than the other with the result that the two rarely have the same appearance in a transverse section. However, they both have the same structure, a ring of nine double filaments with no central filaments.

More distally, the cilia break down to an unordered array of microtubules (Fig. 11D) and, in this region of the scolopidium, the enclosed space has diminished to about 2 μm diameter. The scolopale material usually forms a complete ring around the enclosed space. Distal to this region, the structure is not absolutely clear. The structures that derive from the unordered arrays of loosely-packed tubules have a considerably reduced diameter (Fig. 11E). They retain the enclosing membrane

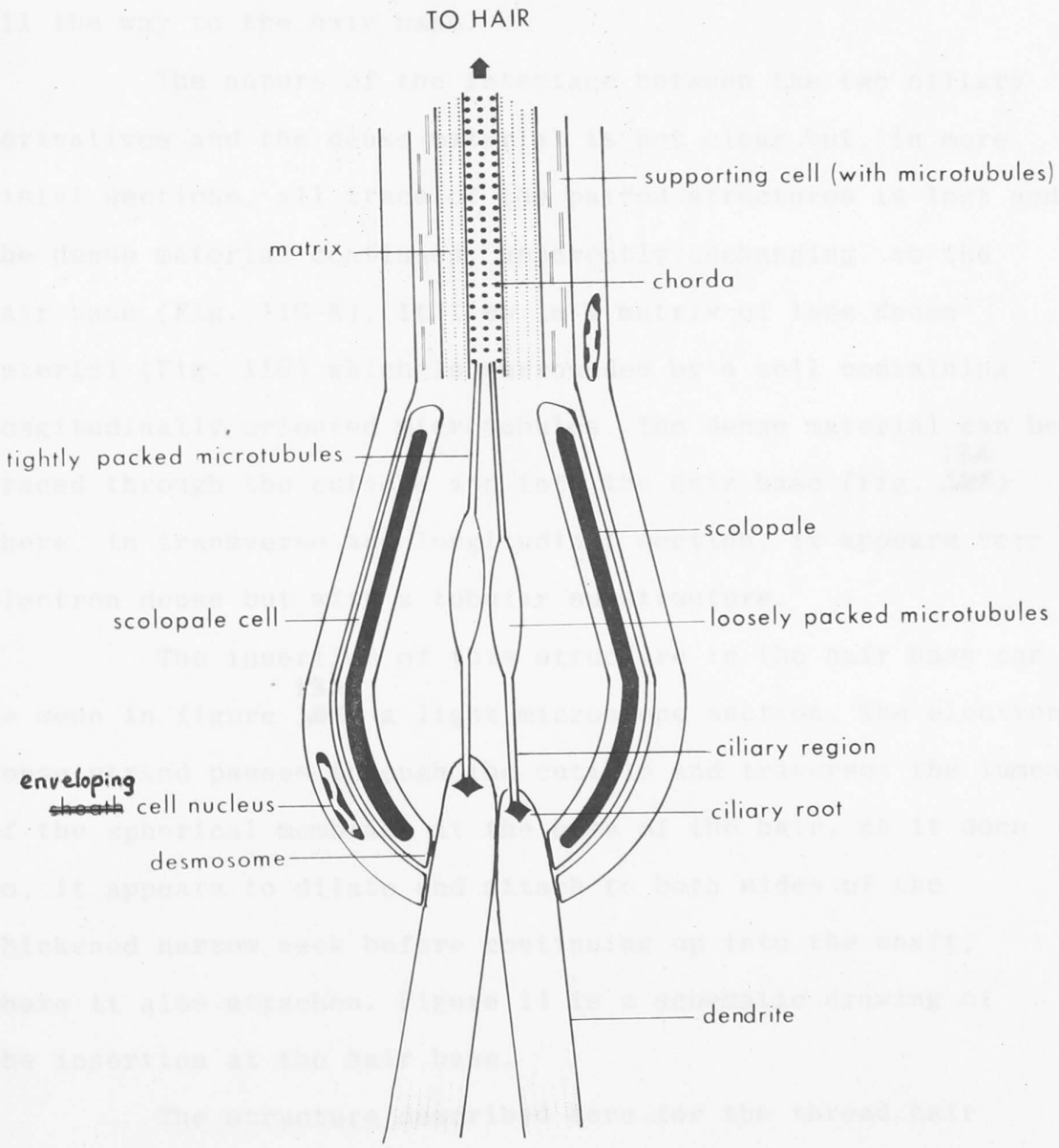


FIG 12 Diagram of proposed scolopidial structure in thread hair receptor system.

and have a dense core and, slightly more distally, they become surrounded by a dense material (Fig. 11F). If the scolopidium is defined by the scolopale material, it terminates about this point, for the scolopale material disappears from the ~~enclosing sheath~~ enveloping cells although the cells themselves are present all the way to the hair base.

The nature of the interface between the two ciliary derivatives and the dense material is not clear but, in more distal sections, all trace of the paired structures is lost and the dense material continues, apparently unchanging, to the hair base (Fig. 11G-K). It lies in a matrix of less dense material (Fig. 11G) which is surrounded by a cell containing longitudinally oriented microtubules. The dense material can be traced through the cuticle and into the hair base (Fig. ~~12A~~ 13A) where, in transverse and longitudinal section, it appears very electron dense but with a tubular substructure.

The insertion of this structure in the hair base can be seen in figure ~~12A~~ 13A, a light microscope section. The electron dense strand passes through the cuticle and traverses the lumen of the spherical membrane at the base of the hair. As it does so, it appears to dilate and attach to both sides of the thickened narrow neck before continuing up into the shaft, where it also attaches. Figure 14 is a schematic drawing of the insertion at the hair base.

The structure described here for the thread hair system of Scylla most closely resembles that described by Schöne and Steinbrecht (1968) for the statolith hairs of the crayfish although, in that system, there are three dendrites per hair instead of two. The ciliary rootlets described earlier closely resemble those found in the crayfish system and the electron-dense strand linking the cilia to the hair base has a direct

Figure 12

The chorda.

A. *Light micrograph of the thread hair base showing the passage of the chorda through the cuticle into the hair (arrows). The narrow neck of the hair (n) is thickened and the chorda appears to attach to the neck on both sides before continuing up the shaft (s).*

B. *Electron micrograph of transverse section of the chorda in the spherical membrane at the base of the hair.*

C. *Electron micrograph of slightly oblique longitudinal section of the chorda inside the spherical membrane at the base of the hair.*

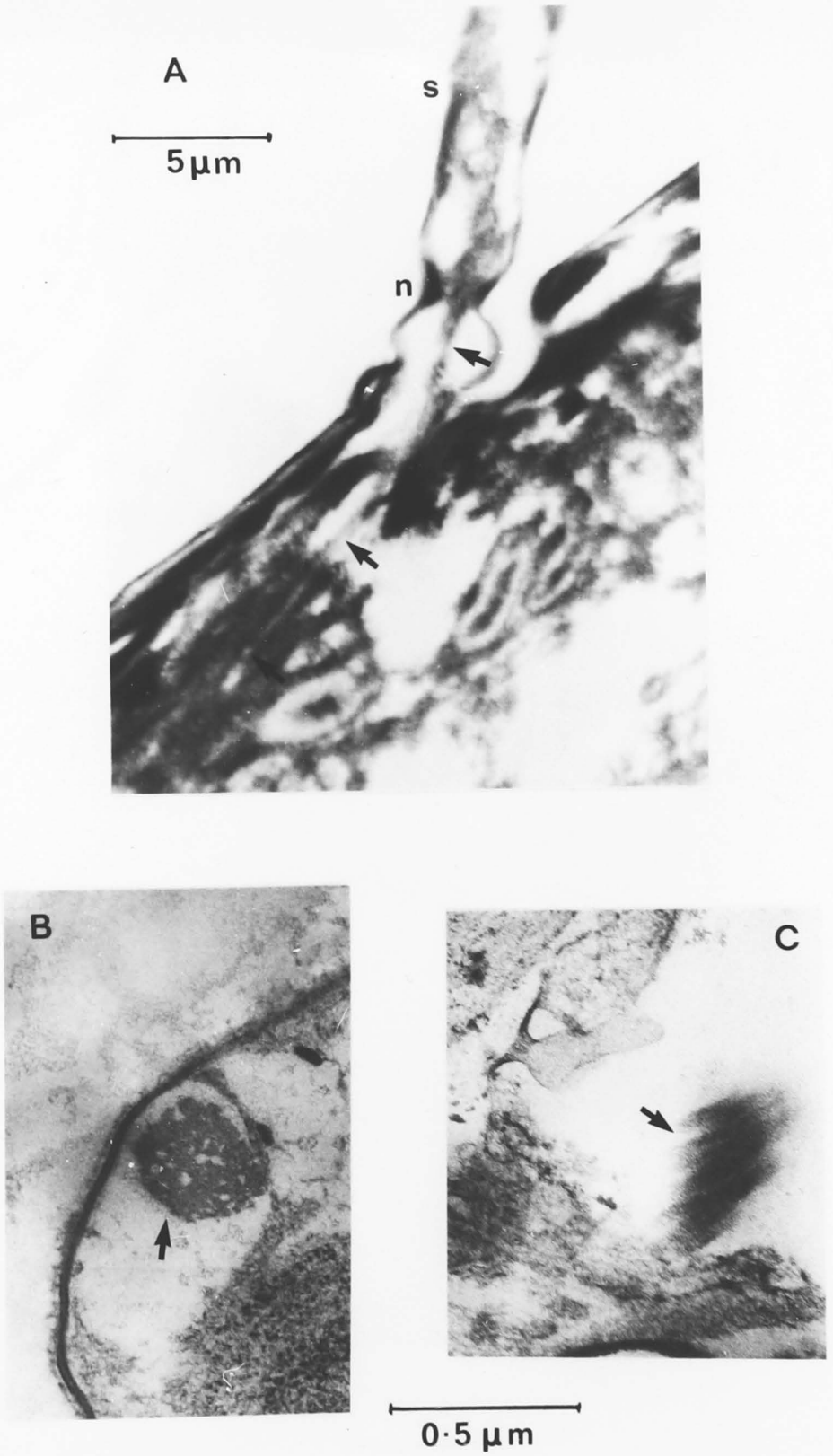


FIG. 13

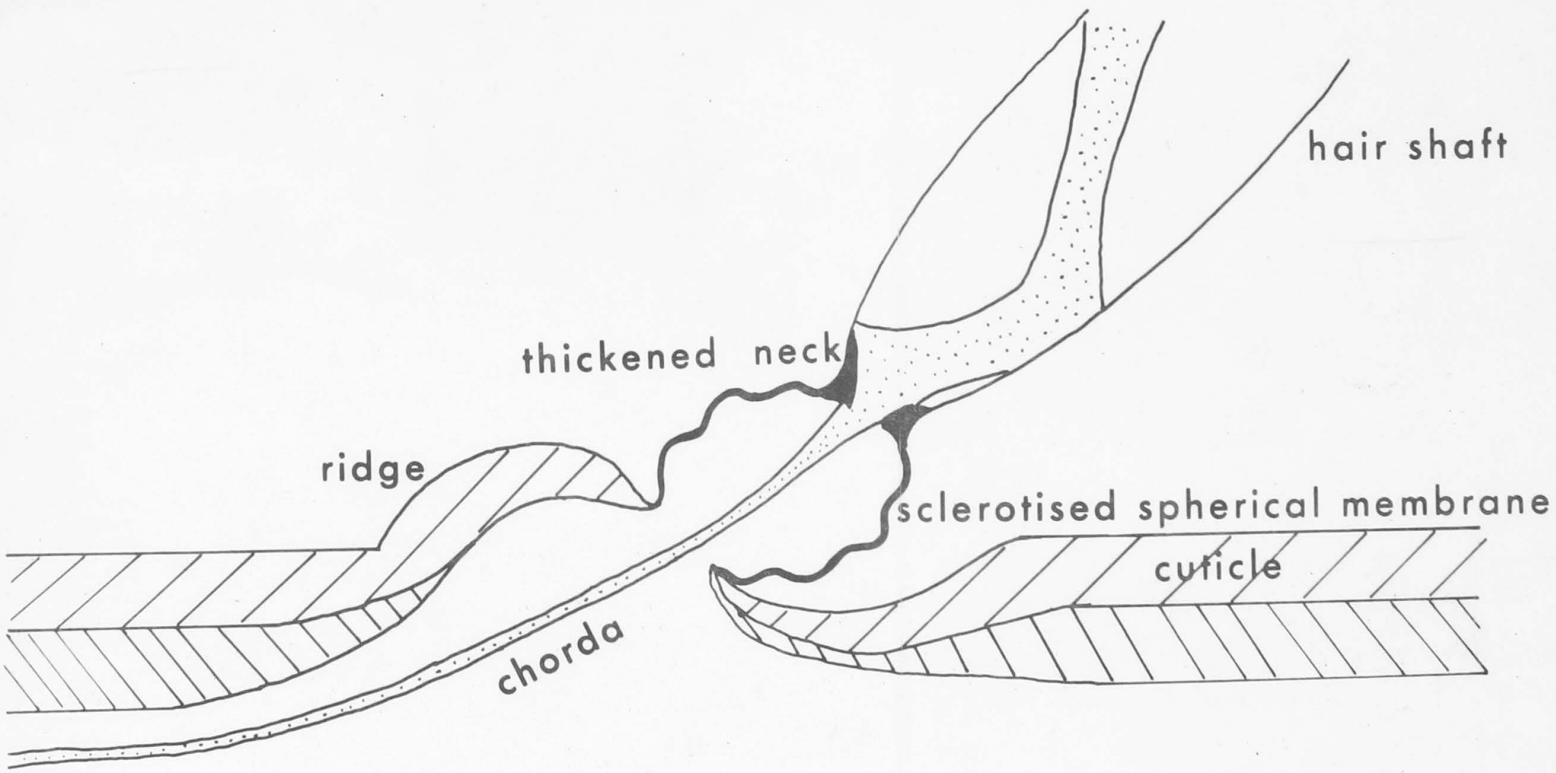


FIG.14 Diagram of proposed insertion of chorda at base of thread hair

equivalent in the chorda of that system. However, there is a difference between the two systems in the insertion of the chorda at the hair base. Schöne and Steinbrecht described the neck of the hair as being sclerotised on only one side, an area they called the "tooth". They described the chorda as inserting inside the hair shaft, on the side opposite the tooth, and on a cuticular spine of the shaft, the "lingula". In Scylla, the neck is thickened on both sides and the chorda attaches to it on both sides, before continuing up the shaft. There is some evidence for cuticular spines in the thread hairs of Scylla from scanning electron microscopy (Fig. 7) but they may have a purely supporting function, keeping the long, thin thread hairs upright.

Schöne and Steinbrecht proposed that, in the system they investigated, the hair acted as a lever on the chorda, the lingula moving up or down when the hair bends and the chorda sliding around the edge of the cuticular pore below the hair base. The counterforce for the system was provided by the attachment of the postciliary segment to the cuticle and the implication was that the adequate stimulus for the receptor system was stretch of the chorda. In the thread hair system of Scylla there is no evidence to support this theory. The chorda does not insert inside the hair shaft on only one side but rather at the thickened neck and on both sides; it will thus not be stretched when the hair bends. Furthermore, the chorda does not make a sharp turn through the cuticle, as described in the crayfish system. The cuticle of the sensory cushion becomes progressively thinner near the hair bases and the chorda follows a gentle curve under the cuticle and up into the base.

Thurm (1964) described the innervation of the hair-

plate receptor in the honey bee. The narrow neck of the hair is filled with a spongy material, thought to be resilin. The nerve terminates in a "tubular body" which is inserted into the spongy material at the neck of the hair. Thurm showed that bending the hair in its preferred plane constricted the neck and squashed the spongy material. The result of this was compression of the tubular body and Thurm showed that this was an adequate stimulus for the sensory nerve, the connection between the two being via a ciliary structure. The transverse and longitudinal sections of the chorda of Scylla in figure 13B, C are almost identical to the comparable sections of Thurm's "tubular body". This has been described as "a number of tubular elements combined in parallel by electron dense material", and a similar structure has also been reported by Moran et al (1971) in the cockroach campaniform sensillum.

In addition, the insertion in Scylla occurs across the neck, as in the hair-plate receptor. No spongy material has been found in the neck of the thread hair but very few sections have been made of the area. It may be absent and the chorda may be compressed directly by flexion of the hair. If it is present, and has the perfect elasticity attributed to resilin, it has important implications concerning the tendency of the thread hairs to revert to their normal position on removal of the stimulus.

In Thurm's system the adequate stimulus is lateral compression of a longitudinally oriented structure, as opposed to longitudinal stretch of such a structure in the system described by Schöne and Steinbrecht. Thurm offered no explanation of how the lateral compression was transmitted down the tubular body to the dendrites but no counterforce would be

required because no stretching of the system is involved. Microtubules have been reported to be involved in rapid transport of proteins (Sjostrand, Frizell and Hasselgren, 1970) and mobility and cell movement (Anderson, Weissman and Ellis, 1966). It is possible, therefore, that lateral compression of the chorda causes either a longitudinal movement of microtubules or a chemical flow down the length of the chorda which, in some way, affects the sensory nerve via a ciliary structure. Moran and Varela (1971) proposed a similar transduction mechanism for the cockroach campaniform sensillum. How this system could incorporate directionality in the response and why two dendrites are required for each hair will be considered in chapter seven after a description of the physiological responses of the thread hair system.

The transduction apparatus of the thread hair in Scylla thus contains many elements common to a wide range of arthropod mechanoreceptor systems. The mechanical transmission of the stimulus, however, seems to resemble more closely an insect system than a crustacean system, although this statement must be tempered by the comment that very few such systems in either class have been fully investigated.

CHAPTER FIVE

Thread Hairs - Dynamic Responses

Introduction

Sandeman and Okajima (1972) recorded from the sensory nerves innervating specific groups of hairs within the statocyst. However, to stimulate the hairs, they opened the statocyst and squirted saline in to create fluid flow. Apart from disturbing the geometry of the statocyst by opening it, they may have been further altering the dynamics of the system by substituting saline for the statolymph.

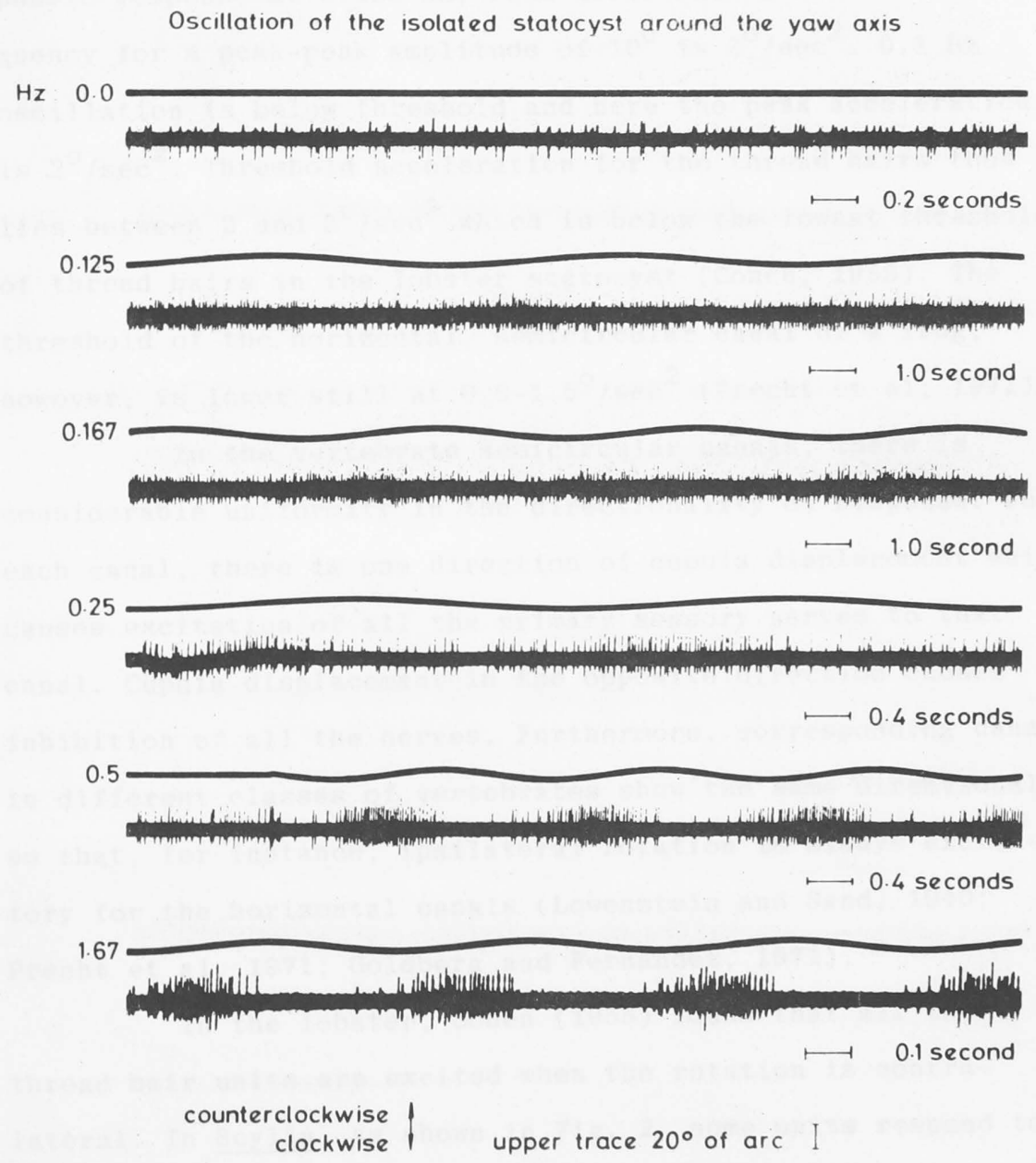
In the experiments to be described here, the statocyst was isolated from the animal but left intact and stimulated over a frequency range compatible with the animal's normal experience. The experimental procedure is described in chapter two.

Electrical Responses of Thread Hairs

Fine bundles of the thread hair nerve were draped over a silver wire electrode and extracellular spikes from one or several units recorded. Most thread hair units have a spontaneous firing level. The statocyst was oscillated sinusoidally at a frequency of about 1 Hz in the horizontal plane and then again in the vertical plane. The majority of units respond to only one or the other of these two stimuli, i.e. rotation in the horizontal or in the vertical plane, and, in this way, the optimum stimulus for each unit was quickly determined.

During sinusoidal oscillation of the statocyst at low frequency, such as 0.25 Hz, the impulse frequency in the thread hair oscillates about the spontaneous level, increasing as the statocyst moves one way and decreasing as it moves the opposite way. (Figure 1). At higher frequencies of oscillation, the change in firing levels from the spontaneous level is greater until, above about 0.5 Hz, there is a burst of activity when

FIG. 1


Oscillation of the isolated statocyst around the yaw axis
Hz 0.0
0.2 seconds
0.125
1.0 second
0.167
1.0 second
0.25
0.4 seconds
0.5
0.4 seconds
1.67
0.1 second
counterclockwise
clockwise
upper trace 20° of arc

the statocyst moves one way and complete inhibition as it moves the other way. As the oscillation frequency increases beyond this, the impulse frequency in the excitatory direction increases but the burst is of shorter duration.

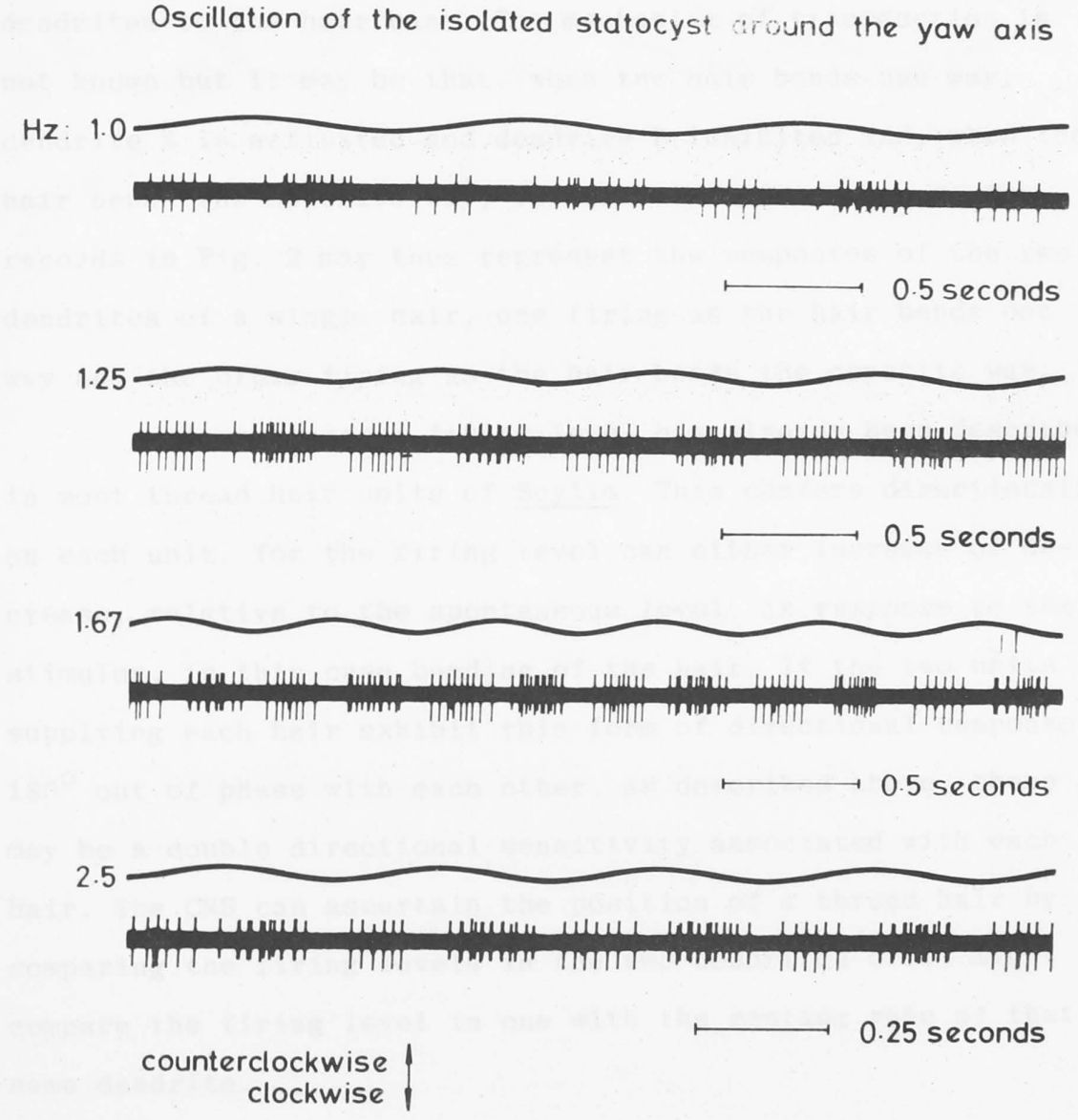
From Figure 1, it can be seen that there is a slight phasic response at 0.125 Hz. Peak acceleration at this frequency for a peak-peak amplitude of 10^{o} is $3^{o}/sec^{2}$. 0.1 Hz oscillation is below threshold and here the peak acceleration is $2^{o}/sec^{2}$. Threshold acceleration for the thread hairs thus lies between 2 and $3^{o}/sec^{2}$ which is below the lowest threshold of thread hairs in the lobster statocyst (Cohen, 1955). The threshold of the horizontal, semicircular canal of a frog, however, is lower still at $0.5\text{-}1.5^{o}/sec^{2}$ (Precht et al, 1971).

In the vertebrate semicircular canals, there is considerable uniformity in the directionality of response. For each canal, there is one direction of cupula displacement which causes excitation of all the primary sensory nerves to that canal. Cupula displacement in the opposite direction causes inhibition of all the nerves. Furthermore, corresponding canals in different classes of vertebrates show the same directionality so that, for instance, ipsilateral rotation is always excitatory for the horizontal canals (Lowenstein and Sand, 1940; Precht et al, 1971; Goldberg and Fernandez, 1971).

In the lobster, Cohen (1955) found that all the thread hair units are excited when the rotation is contralateral. In Scylla, as shown in Fig. 2, some units respond to rotation in one direction while others respond to rotation in the opposite direction.

Rotation in a particular direction is assumed to bend all hairs in one group, upper or lower, in the same

FIG. 2

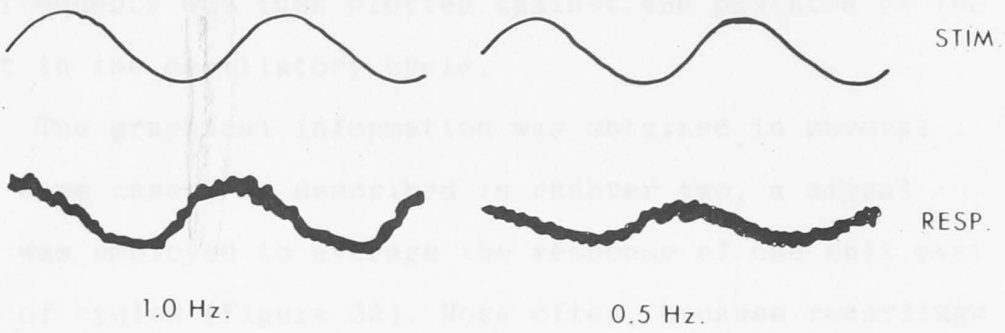

Oscillation of the isolated statocyst around the yaw axis
Hz: 1·0
0·5 seconds
1·25
0·5 seconds
1·67
0·5 seconds
2·5
0·25 seconds
counterclockwise
clockwise

direction. Despite this, when recording solely from one group of hairs, some of the units fire in response to rotation in one direction while other units fire in response to rotation in the opposite direction. The dual innervation of each thread hair may provide an explanation for this observation. Anatomical investigations suggest that a single strand links the two dendrites to the hair base. The mechanism of transduction is not known but it may be that, when the hair bends one way, dendrite A is activated and dendrite B inhibited and, when the hair bends the opposite way, the situation is reversed. The records in Fig. 2 may thus represent the responses of the two dendrites of a single hair, one firing as the hair bends one way and the other firing as the hair bends the opposite way.

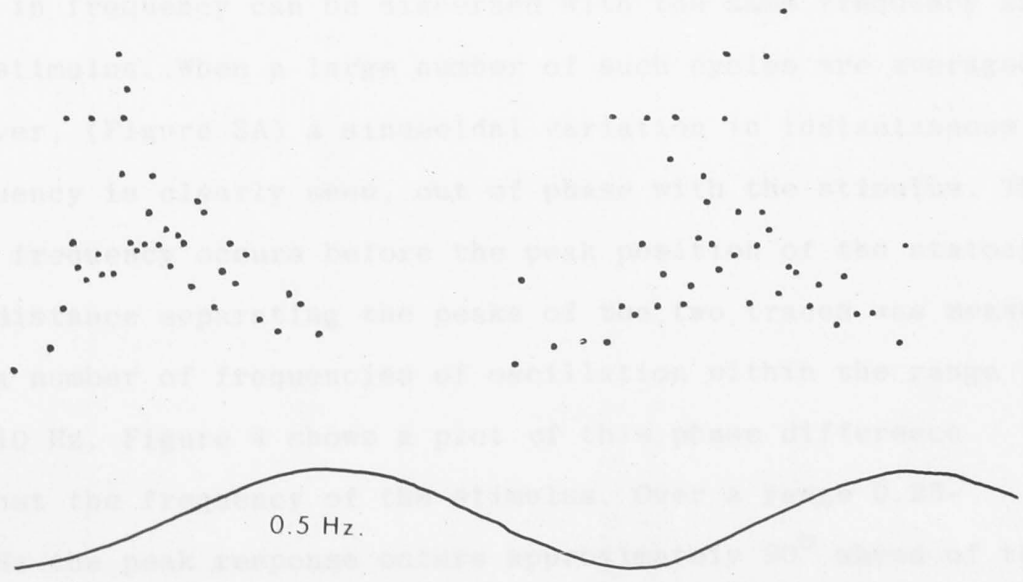
A spontaneous firing level has already been described in most thread hair units of Scylla. This confers directionality on each unit, for the firing level can either increase or decrease, relative to the spontaneous level, in response to the stimulus, in this case bending of the hair. If the two units supplying each hair exhibit this form of directional response, 180° out of phase with each other, as described above, there may be a double directional sensitivity associated with each hair. The CNS can ascertain the position of a thread hair by comparing the firing levels in the two dendrites or it might compare the firing level in one with the resting rate of that same dendrite.

Frequency Analysis

An insight into the dynamic properties of a pendulum-type system such as the thread hairs can be obtained by studying the response of the system to a sinusoidally varying stimulus.



A. Average response over 64 cycles.



B. Computer plot of individual cycles.

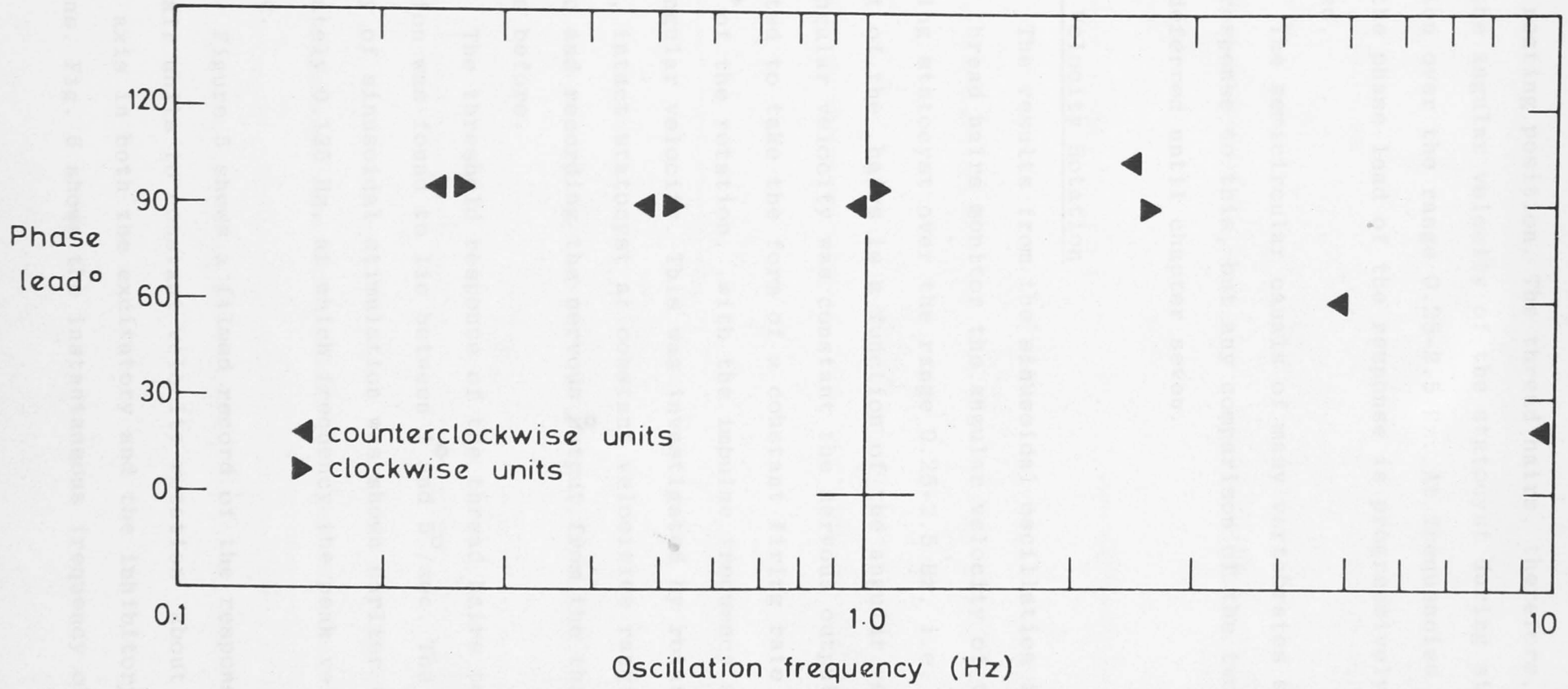
FIG. 3 Instantaneous spike frequency during sinusoidal oscillation of the statocyst.

To this end, the isolated, intact statocyst was oscillated sinusoidally at frequencies ranging from 0.1-10 Hz and the activity of the thread hair nerves recorded. The instantaneous impulse frequency was then plotted against the position of the statocyst in the oscillatory cycle.

The graphical information was obtained in several ways. In some cases, as described in chapter two, a signal analyser was employed to average the response of one unit over a number of cycles (Figure 3A). More often, because recordings from large, single units were rare, a filmed record was made and the interspike intervals measured by hand. These figures were processed by a PDP-II computer to give instantaneous frequency (Figure 3B).

There is some scatter in the instantaneous frequency over a single cycle (Figure 3B) although a rhythmic rise and fall in frequency can be discerned with the same frequency as the stimulus. When a large number of such cycles are averaged, however, (Figure 3A) a sinusoidal variation in instantaneous frequency is clearly seen, out of phase with the stimulus. The peak frequency occurs before the peak position of the statocyst. The distance separating the peaks of the two traces was measured for a number of frequencies of oscillation within the range 0.1-10 Hz. Figure 4 shows a plot of this phase difference against the frequency of the stimulus. Over a range 0.25-2.5 Hz the peak response occurs approximately 90^{o} ahead of the peak position. The response is thus in phase with the angular velocity of the statocyst, which follows a sinusoidal course 90^{o} ahead of the position of the statocyst. It is known (Sandeman and Okajima, 1972) that the discharge rate of the nerves is a function of the displacement of the thread hairs

FIG. 4


120
90
60
30
0
Phase lead °
counterclockwise units
clockwise units
0·1
1·0
10
Oscillation frequency (Hz)

from the resting position. The thread hairs, therefore, monitor the angular velocity of the statocyst during sinusoidal oscillation over the range 0.25-2.5 Hz. At frequencies above 2.5 Hz, the phase lead of the response is progressively diminished.

The semicircular canals of many vertebrates show a similar response to this, but any comparison of the two systems will be deferred until chapter seven.

Constant Velocity Rotation

The results from the sinusoidal oscillation indicate that the thread hairs monitor the angular velocity of the oscillating statocyst over the range 0.25-2.5 Hz, i.e. the displacement of the hairs is a function of the angular velocity. If the angular velocity was constant the nervous output would be expected to take the form of a constant firing rate for the duration of the rotation, with the impulse frequency a function of the angular velocity. This was investigated by rotating the isolated, intact statocyst at constant velocities ranging from 1-60^{o}/sec and recording the nervous output from the thread hairs, as before.

The threshold response of the thread hairs to such stimulation was found to lie between 1^{o} and 5^{o}/sec. The threshold frequency of sinusoidal stimulation was shown earlier to be approximately 0.125 Hz, at which frequency the peak velocity is 4^{o}/sec.

Figure 5 shows a filmed record of the response of thread hair units to constant velocity rotation about a vertical axis in both the excitatory and the inhibitory directions. Fig. 6 shows the instantaneous frequency of a

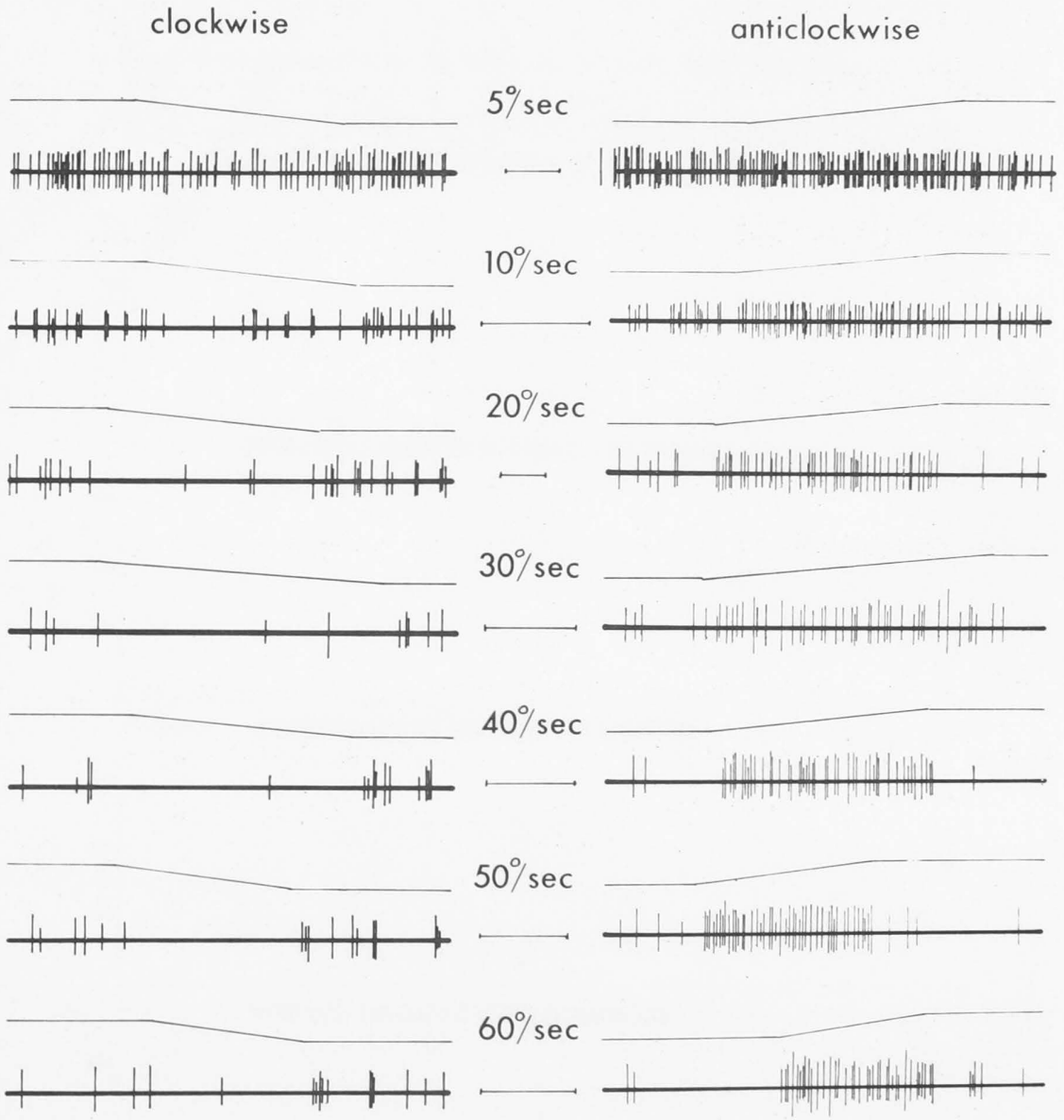
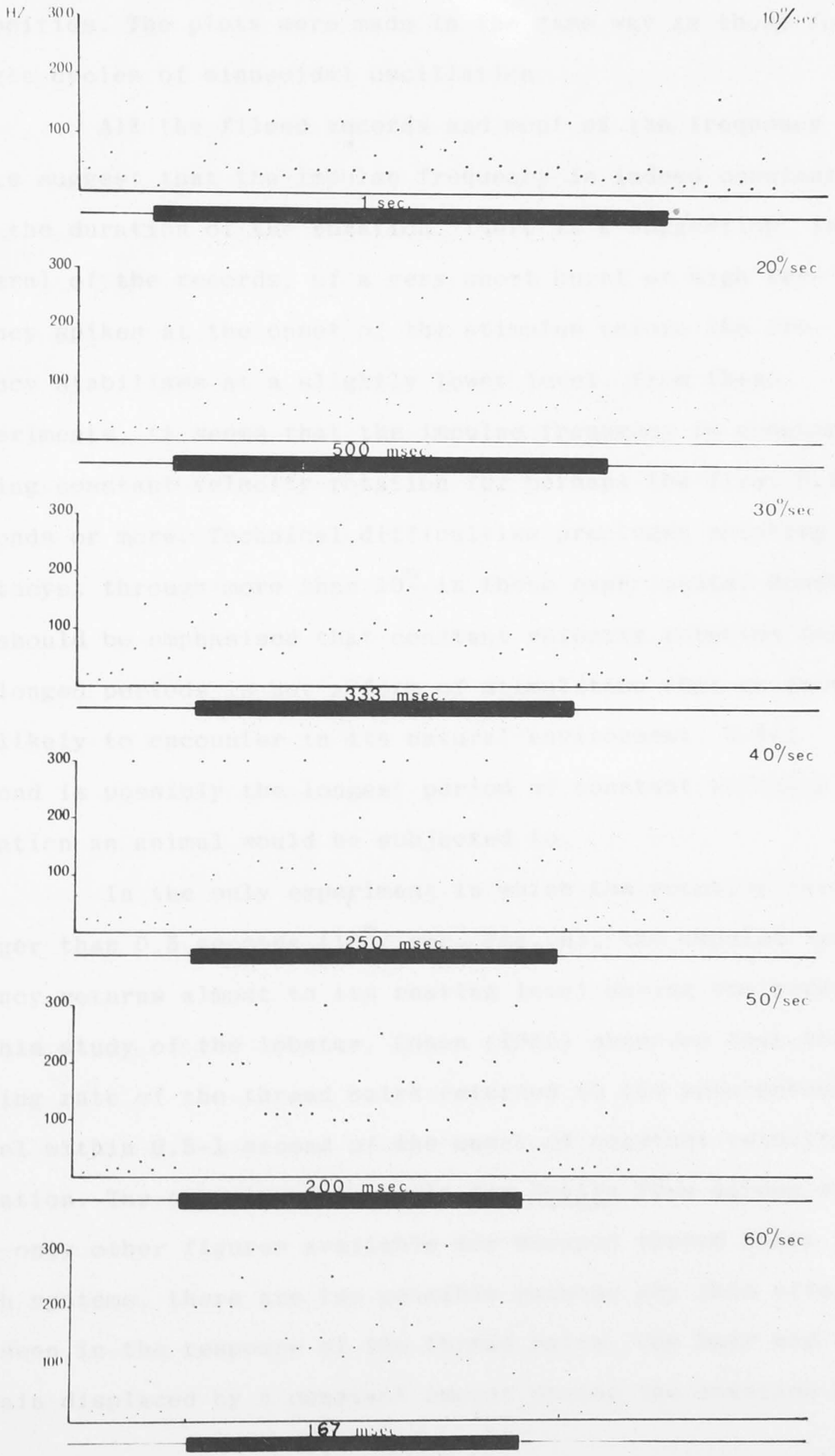


FIG. 5 Thread hair responses to rotation at constant velocity in the horizontal plane. Stimulus marker indicates period of movement. Calibration is 0.5 sec. for 5 and 10, 0.1 sec. the rest.

Figure 6

Instantaneous frequency of a single thread hair unit during rotation at constant velocity (black bar).

FIG. 6


Hz
300
200
100
10°/sec
1 sec.
20°/sec
500 msec.
30°/sec
333 msec.
40°/sec
250 msec.
50°/sec
200 msec.
60°/sec
167 msec.

single thread hair unit during rotation of the statocyst about a vertical axis in the excitatory direction over a range of velocities. The plots were made in the same way as those for single cycles of sinusoidal oscillation.

All the filmed records and most of the frequency plots suggest that the impulse frequency is indeed constant for the duration of the rotation. There is a suggestion, in several of the records, of a very short burst of high frequency spikes at the onset of the stimulus before the frequency stabilises at a slightly lower level. From these experiments, it seems that the impulse frequency is constant during constant velocity rotation for perhaps the first 0.5 seconds or more. Technical difficulties precluded rotating the statocyst through more than 10^{o} in these experiments. However, it should be emphasised that constant velocity rotation for prolonged periods is not a form of stimulation that an animal is likely to encounter in its natural environment. 0.5-1 second is possibly the longest period of constant velocity rotation an animal would be subjected to.

In the only experiment in which the rotation lasted longer than 0.5 seconds (10^{o}/ sec. Fig. 6), the impulse frequency returns almost to its resting level during the rotation. In his study of the lobster, Cohen (1955) observed that the firing rate of the thread hairs returned to its spontaneous level within 0.5-1 second of the onset of constant velocity rotation. The experimental result for Scylla thus agrees with the only other figures available for decapod thread hairs. In both systems, there are two possible reasons why this effect is seen in the response of the thread hairs. The hair may remain displaced by a constant amount during the rotation but

the sensory nerve may show adaptation to the mechanical stimulus. The second possiblity is that the hair may have an inherent elasticity and will tend to slowly return to the resting position during rotation. This has been shown to be an important factor in the behaviour of the cupula in the vertebrate semicircular canal.

Steinhausen (1931) studied the cupula of the pike and observed its behaviour during rotational stimulation. He rotated the animal for a prolonged period at a constant velocity of 180°/sec and observed that the cupula, after being displaced extensively from its zero position by the initial acceleration, reverted to its zero position over an exponential time course lasting 20 seconds. In this way, he demonstrated the elasticity of the cupula and its inherent tendency to revert to a zero position. In other experiments, he was also able to show that the time required to return to zero was a function of the displacement from zero and ranged from 1-60 seconds.

Lowenstein and Sand(1940) studied the electrophysiological responses of the semicircular canals of the elasmobranch Raja to various stimuli including constant velocity rotation. Using a considerably slower speed than Steinhausen, they found that the impulse frequency returned to the spontaneous level 20-30 seconds after the onset of constant velocity. On the basis of their results and the earlier ones of Ross (1936), Lowenstein and Sand concluded that the observed physiological response to constant velocity rotation was caused mainly by the elastic properties of the cupula and that nervous adaptation played little or no part.

Cohen (1960) came to a similar conclusion about the thread hairs of the lobster statocyst, after measuring the

elasticity of the hairs and the adaptation rate of the nerves. In Scylla, the question cannot be answered from the results presented so far but it will be considered again in chapter six, where the effect of gravity on the thread hairs is described.

Upper and Lower Thread Hairs

By carefully scraping away the dendritic connections on the sensory cushion, it was possible to selectively record from the upper group of thread hairs in the common canal or the lower group at the bottom of the vertical canal. In this way, the sensitivity of each group to rotation in different planes could be ascertained.

The lower thread hairs respond to movement in the vertical plane (pitch and roll) but not to movement in the horizontal plane (yaw). The upper hairs show the reverse sensitivity, responding to yaw but not to pitch, although a phasic response to sinusoidal pitching movements has been recorded at 5 Hz (Fig. 7).

Sandeman and Okajima (1972) injected dye into the statocyst and showed that there was some fluid movement in the vertical canal during yaw movements. However, it seems that most of the flow is around the horizontal canal and it may be that the flow into the vertical canal is so small that the lower hairs would not be stimulated. Thus, the insensitivity of the lower thread hairs to yaw is not difficult to understand. On the other hand, the upper thread hairs are located in the common canal, which, as the name implies, is assumed to form an arm of both the vertical and horizontal canals. Fluid flows in the horizontal canal (yaw) and the vertical canal (pitch)

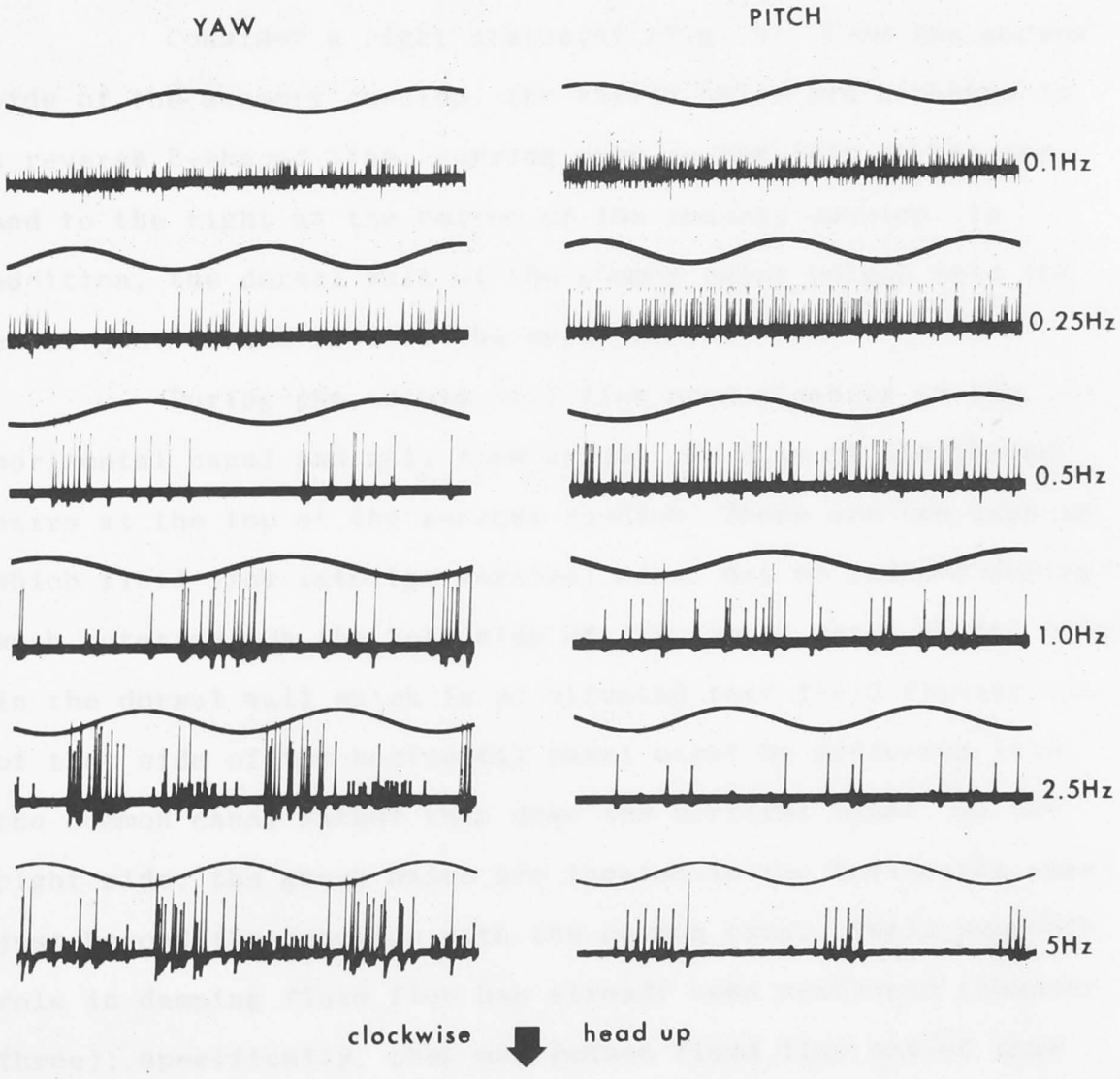


FIG. 7 Response of upper thread hairs to pitch and yaw.

should both involve the common canal and thus stimulate the thread hairs. This has been shown not to be the case, no response being elicited by pitching movements. Explanation of these results requires a re-assessment of the internal architecture of the statocyst and of probable fluid flows during pitch and yaw.

Consider a right statocyst (Fig. 8): From the convex side of the sensory cushion, the thread hairs are arranged in a reverse S-shaped line, curving away to the left at the top and to the right at the bottom of the sensory cushion. In addition, the dorsal wall of the common canal bulges into the canal just to the left of the upper hairs.

During yaw, fluid will flow predominantly in the horizontal canal and will flow across the line of the thread hairs at the top of the sensory cushion. There are two ways in which fluid flow into the vertical canal may be reduced during such rotation. On the left side of the common canal is the bulge in the dorsal wall which is so situated that fluid flowing out of that side of the horizontal canal might be deflected into the common canal rather than down the vertical canal. On the right side, the group hairs are located in the horizontal canal, just beyond the junction with the common canal. Their possible role in damping fluid flow has already been mentioned (chapter three); specifically, they may reduce fluid flow out of that side of the horizontal canal into the vertical canal.

During pitch, fluid will flow predominantly around the periphery of the sensory cushion. The lower hairs are so grouped that such flow, in either direction, will be at right-angles to the line of hairs. However, anti-clockwise fluid flow would appear to run along the curving line of the upper thread hairs rather than across it. Clockwise fluid flow, which

Figure 8

Diagram to show how upper thread hairs may discriminate between pitch and yaw. Figure shows sensory cushion of right statocyst from convex (inside) surface and arrangement of thread hairs. Small arrows indicate rotation of statocyst, large ones projected fluid flows. In pitch (or roll), fluid flows in the vertical canal around the periphery of the sensory cushion. Fluid flows across the line of lower thread hairs but may flow along the line of upper hairs, causing little or no bending thereof. In yaw, fluid flows predominantly in the horizontal canal (projecting out of the page at right angles). Fluid leaving the horizontal canal and entering the common canal will tend to flow across the line of upper hairs, causing maximum bending.

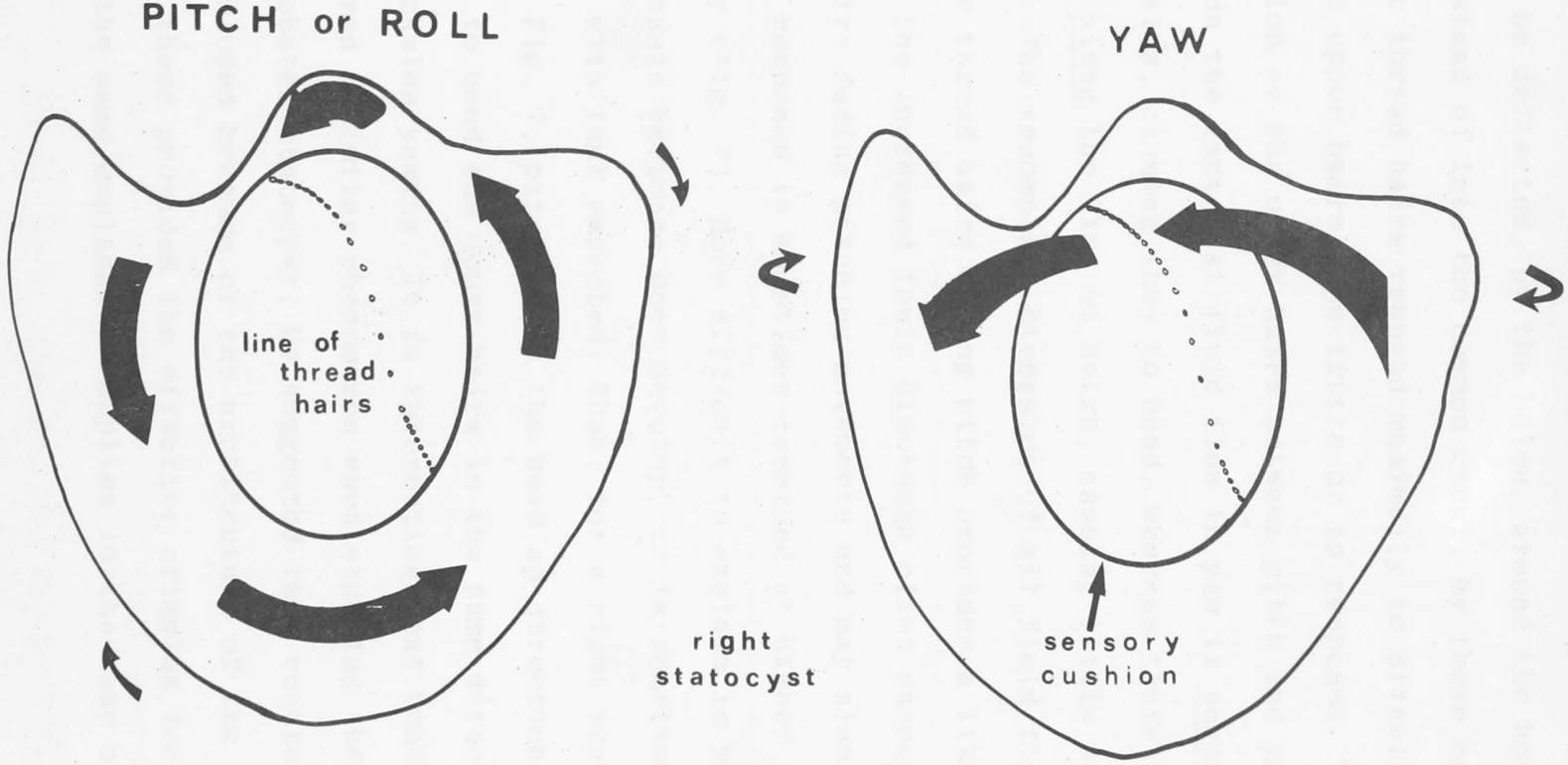

PITCH or ROLL
YAW
line of
thread
hairs
right
statocyst
sensory
cushion

FIG. 8

might be expected to have a greater effect on the upper hairs, may well be deflected, by the bulge, around the horizontal canal instead of into the common canal. By these means, perhaps, the lower thread hairs respond maximally to pitching movements while the upper hairs show little or no response. The discrimination by the upper hairs between pitch and yaw thus depends on the fact that fluid flow in yaw is across the line of the hairs, causing them to bend, whereas fluid flow during pitch is along the line of hairs, causing little or no bending.

The incomplete diversion of all fluid flows away from the upper thread hairs during pitch provides a likely explanation for the increased tonic discharge often encountered from these hairs during pitching movements and may also explain why a phasic response is sometimes recorded at higher stimulus intensity (Fig. 7). More difficult to explain is why, when such a phasic response does develop, it is sometimes 180^{o} out of phase with that expected. Thus, for a right statocyst, as shown in Fig. 7, pitching in the head up direction would be expected to bend the upper hairs in the same direction as anti-clockwise yawing. It is interesting that Cohen (1960) encountered a similar phenomenon when studying the thread hairs of the lobster statocyst. He suggested that countercurrents were developed because of the architecture of the statocyst and that these provided the effective stimulus for the hairs. Perhaps the same explanation applies in the case of Scylla.

Vibration Receptors

Large amplitude units are sometimes recorded in statocyst nerves, showing a low, irregular, spontaneous firing level and often silent for long periods. The only effective

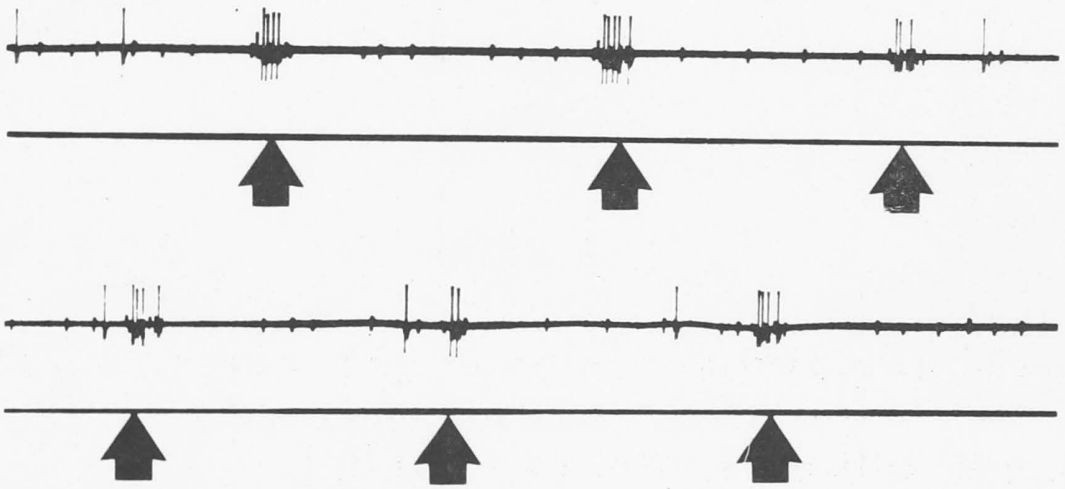


FIG. 9 Large amplitude unit that responds only to a light tap on the apparatus (arrows)

stimulus for such a unit is a light tap on the apparatus which produces a brief, high frequency burst (Fig. 9). The spikes are larger than for any other unit and, in this and other respects, they are very like Cohen's (1955) vibration receptors in the lobster statocyst.

The units were always encountered while looking for thread hair units and are, therefore, associated with the thread hair nerve, but no effort was made to trace the receptors.

CHAPTER SIX

Thread Hairs - Static Responses

Introduction

In the vertebrate vestibular labyrinth, the semicircular canals function as rotary accelerometers and the otolith organs respond to linear accelerations, including that of gravity. Until recently, it was thought that there was no overlap in the sensitivities of these two components of the vestibular system, that is, the semicircular canals were insensitive to linear accelerations and the otolith organs were insensitive to angular acceleration. There were reservations on theoretical grounds about the otolith organs because their construction should render them susceptible to angular accelerations. It has now been shown that they are, in fact, susceptible to such stimulation but that their construction imposes upon them a limited frequency response, so that their role as rotary accelerometers is restricted (Lowenstein, 1972). The insensitivity of the semicircular canals to linear acceleration was unchallenged, except for a report by Ledoux (1949). He reported that the response of a frog's semicircular canal to a standard rotational stimulus was affected by the position of the animal's head and that the spontaneous firing level of the canal was similarly affected. The report was largely ignored although Lowenstein (1972) later reported that he had observed a similar effect in the elasmobranch many years before (Lowenstein and Sand, 1936, 1940) but had not attributed to it any true physiological significance. In the last decade, the susceptibility of the semicircular canals to linear acceleration has been calculated and modelled (Steer, 1967) and demonstrated (Lowenstein, 1972).

In the invertebrates, Budelmann and Wolff (1973) were the first to demonstrate conclusively a gravitational response

in the rotation receptors of a statocyst. In Octopus vulgaris, recording from hairs that monitor rotation about the horizontal axes, they showed that the response to such rotation varied with the starting position of the animal about the horizontal axis. The octopus, like the vertebrates, has a cupula which envelopes the sensory hairs and increases the surface area exposed to fluid flow. In both the vertebrate and the octopus, the hypothesis that the cupula is more dense than the canal fluid has been advanced to explain the effects of gravity on the rotation receptors (Budelmann and Wolff, 1973; Lowenstein, 1972).

In his study of the lobster statocyst, Cohen (1955) reported that the spontaneous firing rate of the thread hair nerves was the same, whatever the orientation of the animal, and that the response to a standard rotational stimulus was the same. No cupula-like structure has ever been seen to envelope the thread hairs of a decapod crustacean statocyst and the possibility arises that any position sensitivity of rotation receptors is confined to those systems possessing a cupula. Despite this possibility, the thread hairs of Scylla were investigated for their response to gravity. The thread hairs were observed directly during tilting of the statocyst and the activity of their nerves was recorded with the statocyst in different orientations.

Directly Observed Movements of the Thread Hairs in Response to Gravity

In order to investigate the effects of gravity on them, the thread hairs were observed directly as the sensory cushion was rotated through 360^{o}.

The sensory cushion was carefully excised from a statocyst. Using a tiny smear of contact adhesive, it was attached to the fire-polished end of a small glass rod. Enough of the walls of the vertical canal around the sensory cushion were left to provide points of attachment so that no part of the sensory cushion was in contact with the glue, and the cushion remained undisturbed. The rod measured approximately 5 mm diameter and 5 cm length. The viewing chamber was a glass cuvette from a spectrophotometer, measuring, 1 x 1 x 4 cm. A tight-fitting perspex stopper was machined to fit the cuvette and then a hole drilled in this stopper to form a tight fit around the glass rod. Both seals were completed with silicone vacuum grease. The sensory cushion could thus be suspended in a sealed chamber of saline, the thread hairs projecting out into the fluid. As the glass rod was circular, it could be both rotated and moved longitudinally in the stopper so that the desired orientation of the sensory cushion was achieved.

The cuvette was mounted longitudinally on an aluminium plate measuring 1 x 30 x 45 cm. Also mounted on the plate were a binocular microscope and two microscope lamps; these latter were fitted with heat filters. In this way, with the sensory cushion mounted vertically, the thread hairs, projecting horizontally into the fluid, could be viewed directly from above.

The plate, supporting the microscope and cuvette, was mounted on a spindle and this was supported by bearings on top of a cage of angle iron measuring approximately 75 x 75 x 75 cm. The plate could thus be rotated through 360^{o} and held at any point in that orbit and the same view of the sensory cushion obtained each time (Fig. 1).

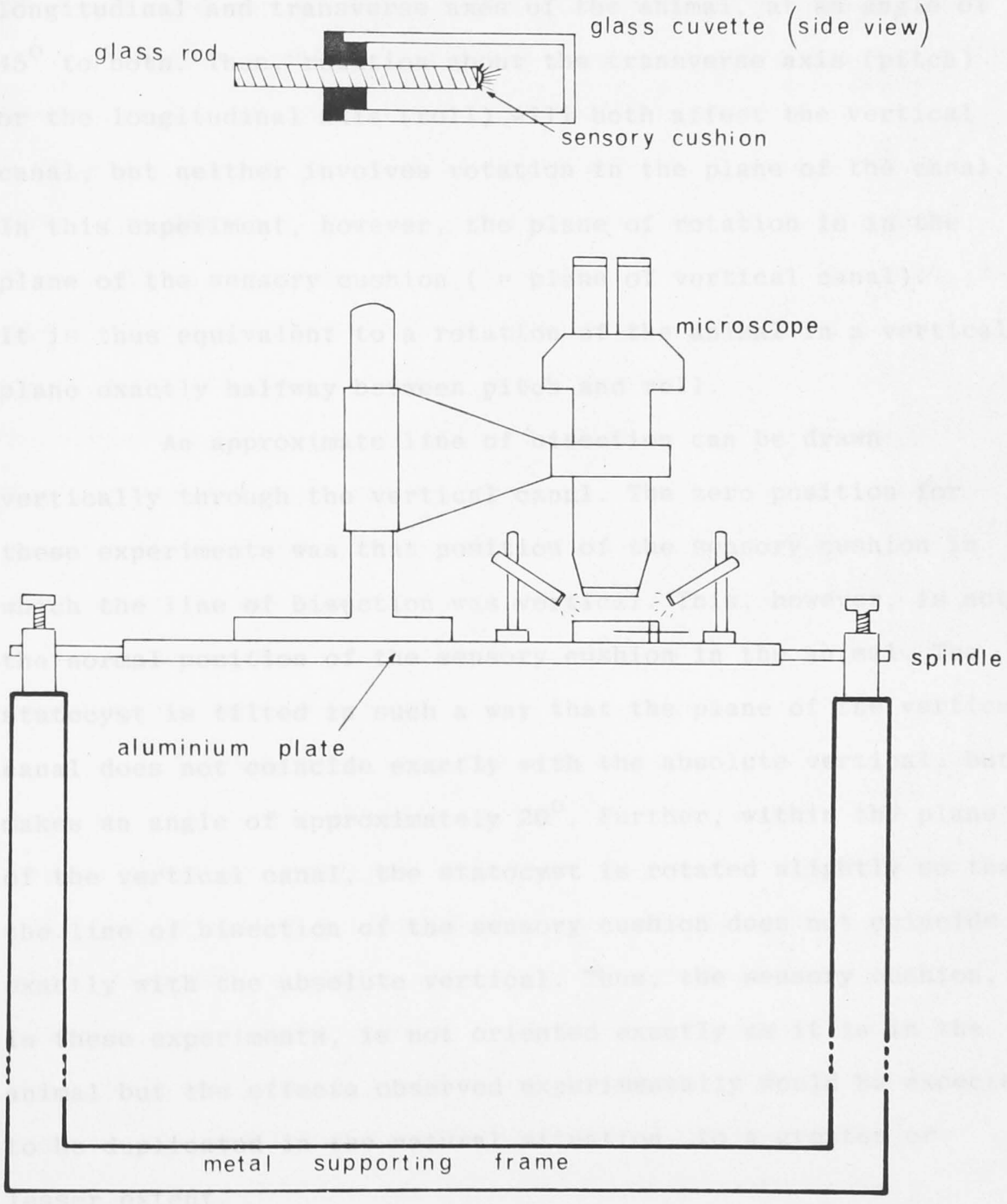


FIG. 1
(See text for dimensions)

The sensory cushion constitutes a major part of the vertical canal of the statocyst. The circumferential plane of the vertical canal lies between the vertical planes of the longitudinal and transverse axes of the animal, at an angle of 45^{o} to both. Thus, rotation about the transverse axis (pitch) or the longitudinal axis (roll) will both affect the vertical canal, but neither involves rotation in the plane of the canal. In this experiment, however, the plane of rotation is in the plane of the sensory cushion (= plane of vertical canal). It is thus equivalent to a rotation of the animal in a vertical plane exactly halfway between pitch and roll.

An approximate line of bisection can be drawn vertically through the vertical canal. The zero position for these experiments was that position of the sensory cushion in which the line of bisection was vertical. This, however, is not the normal position of the sensory cushion in the animal. The statocyst is tilted in such a way that the plane of the vertical canal does not coincide exactly with the absolute vertical, but makes an angle of approximately 20^{o}. Further, within the plane of the vertical canal, the statocyst is rotated slightly so that the line of bisection of the sensory cushion does not coincide exactly with the absolute vertical. Thus, the sensory cushion, in these experiments, is not oriented exactly as it is in the animal but the effects observed experimentally would be expected to be duplicated in the natural situation, to a greater or lesser extent.

The plate was rotated both clockwise and anticlockwise and both upper and lower groups of hair were observed. Since the extreme upper and lower hairs project almost vertically, these were not easy to see with the microscope and hairs in the

central 70% of the cushion contributed the bulk of the results.

The angle of the thread hair was measured in the following way: One eyepiece of the microscope held a 10 x 10 grid and this was fixed in a position parallel to the surface of the sensory cushion. The other eyepiece held a single diametric line which could be rotated. The microscope was focused on a hair or group of hairs and the eyepiece rotated until the diametric line was parallel to the hair. The superposition, in the focal plane of the microscope, of the image of the straight line graticule on the image of the grid in the other eyepiece gave an angle, which could be calculated trigonometrically. There is an element of error in the measurements due to parallax problems but this is difficult to estimate without polar plots of the hair's response to tilting. The predominant plane of movement of the hair is known to be at rightangles to the line of hairs and it is in this plane that the measurements were made.

The plate was rotated slowly through 20^{o} steps and then left for three minutes before any reading was taken. The thread hairs are extremely sensitive to angular rotation and even the smoothest motion involves an initial acceleration from zero. In addition, there is a slight deceleration when the rotation stops which results in a brief continued fluid movement, with consequent movement of the hairs. Sufficient time must be allowed for all fluid movements, and hair movements resulting therefrom, to cease. After three minutes, the main forces acting on the thread hair are assumed to be gravity and the hair's own elasticity.

The results were plotted as the total angle moved by the hair either side of the zero position for each new

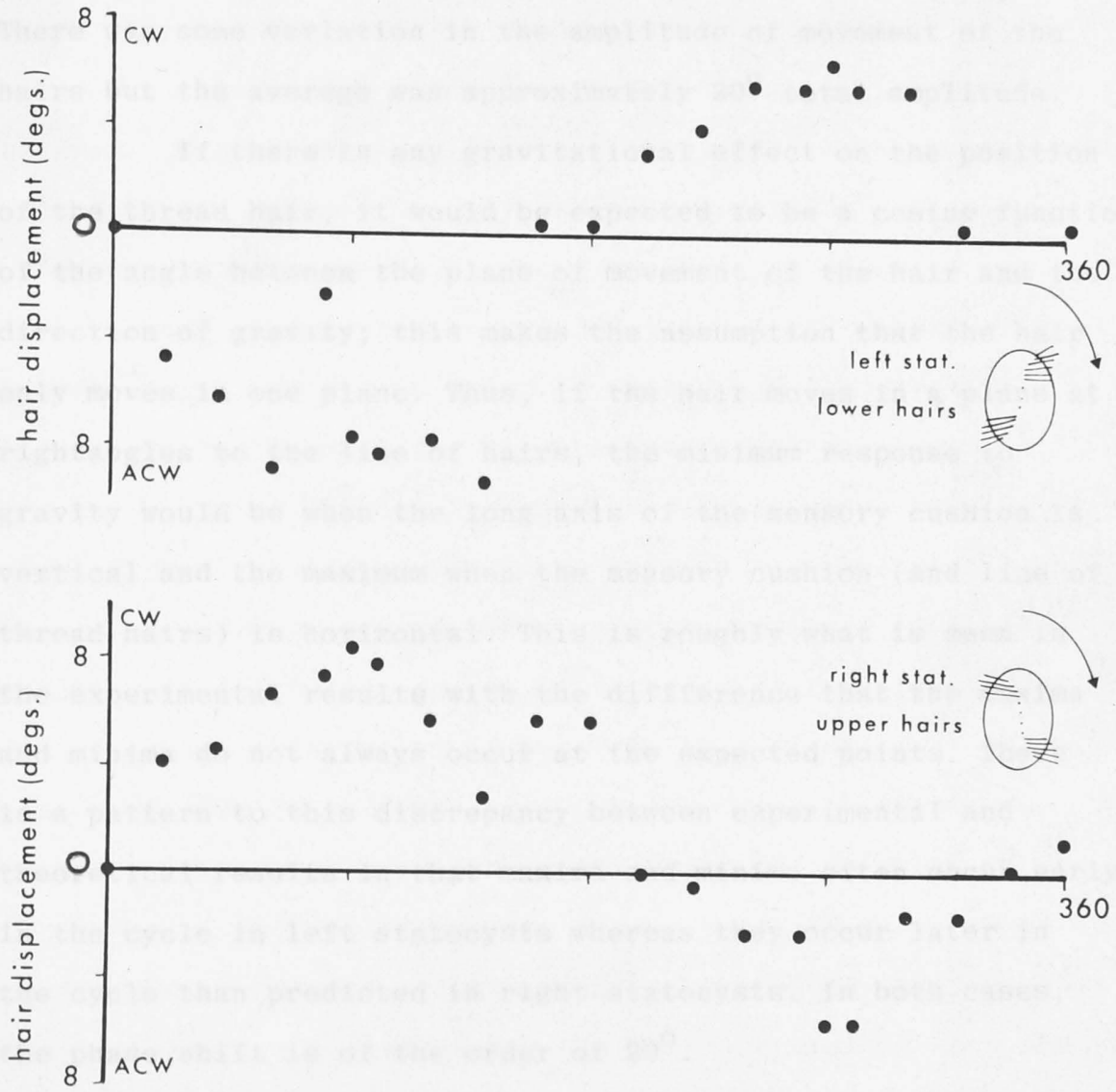


FIG. 2 Two examples of the displacement of thread hairs during clockwise rotation of the sensory cushion.

position of the sensory cushion in the orbit (Fig. 2). The hairs can be clearly seen to assume different positions when the sensory cushion alters its position with respect to gravity. There was some variation in the amplitude of movement of the hairs but the average was approximately 20^{o} total amplitude.

If there is any gravitational effect on the position of the thread hair, it would be expected to be a cosine function of the angle between the plane of movement of the hair and the direction of gravity; this makes the assumption that the hair only moves in one plane. Thus, if the hair moves in a plane at rightangles to the line of hairs, the minimum response to gravity would be when the long axis of the sensory cushion is vertical and the maximum when the sensory cushion (and line of thread hairs) is horizontal. This is roughly what is seen in the experimental results with the diffference that the maxima and minima do not always occur at the expected points. There is a pattern to this discrepancy between experimental and theoretical results in that maxima and minima often occur early in the cycle in left statocysts whereas they occur later in the cycle than predicted in right statocysts. In both cases, the phase shift is of the order of 20^{o}.

This observation may be explained by the fact that the hairs in question are not at their mean position when the sensory cushion is in its zero position. In the theoretical zero position the line of thread hairs is vertical and the gravitational effect is zero. In the experimental zero position, however, the central thread hairs form an approximately vertical line but the hairs at the end form a curving line to the left at the top and the right at the bottom for a right statocyst as shown in Fig. 3. This means that there is actually a

gravitational force acting on the upper and lower hairs when the sensory cushion is supposedly in the zero position. If a right sensory cushion is rotated clockwise, the line of upper hairs only becomes vertical after about 20^{o} rotation; consequently, the line is only horizontal after 110^{o} rotation so the maximum response occurs approximately 20^{o} late. By the same argument, the minimum is only achieved after 200^{o} rotation, after which point the hairs begin to bend the opposite way; the crossover point thus occurs 20^{o} late. The S-shaped distribution of the hairs on a sensory cushion may thus provide a structural explanation for the observed results.

Figure 3 shows how a 20^{o} clockwise rotation of a right sensory cushion would have the effect of bringing both upper and lower groups of hairs into the vertical, i.e. the position in which they are least affected by gravity. A similar anticlockwise rotation would have the same effect in a left statocyst. The bulk of the thread hairs occur in these end groups so that such rotations might bring the majority of the thread hairs in both statocysts into a position where they were unaffected by gravity. It is known, as described earlier, that the statocyst is tilted inside the basal segment so that the horizontal canal is not exactly horizontal and the vertical canal is actually slightly out of the vertical. Measurements indicate that these tilts of both statocysts in the natural situation are of appropriate magnitude and direction to achieve the arrangement of thread hairs described. In other words, in the natural position of the statocysts, the upper and lower thread hairs may be aligned with the vertical, unaffected by gravity and, therefore, at their most sensitive for angular accelerations.

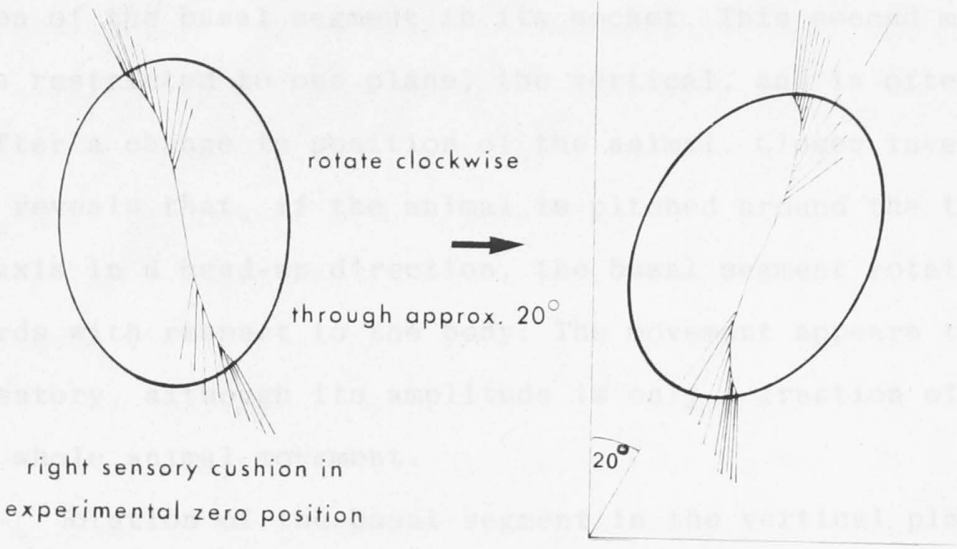


FIG. 3 Diagram to show how 20° tilt of statocyst might bring the densely-grouped upper and lower hairs into the vertical plane

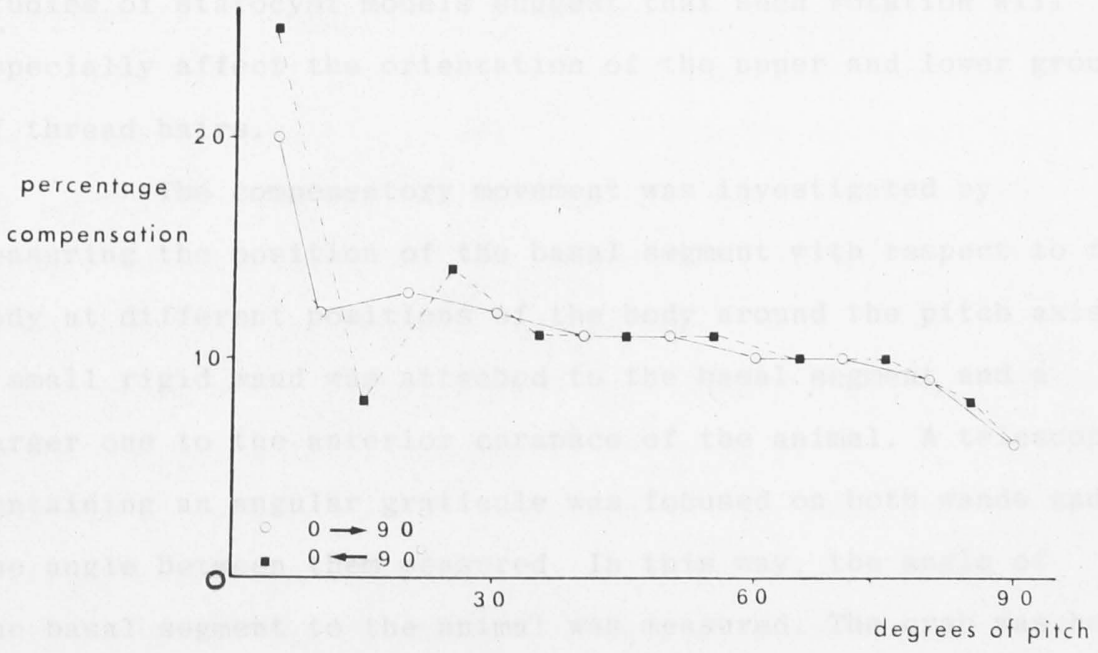


FIG. 4 Compensatory movement of antennule when animal is pitched through 90° from the zero position. The curves for forward and backward pitch are the same.

Compensatory Movement of Antennules

The crab constantly waves the distal segments of the antennules and, less frequently, can be seen to alter the position of the basal segment in its socket. This second movement is restricted to one plane, the vertical, and is often seen after a change in position of the animal. Closer investigation reveals that, if the animal is pitched around the transverse axis in a head-up direction, the basal segment rotates downwards with respect to the body. The movement appears to be compensatory, although its amplitude is only a fraction of that of the whole animal movement.

Rotation of the basal segment in the vertical plane will inevitably cause rotation of the statocyst and thus alter its orientation with respect to the various axes of the animal. Studies of statocyst models suggest that such rotation will especially affect the orientation of the upper and lower groups of thread hairs.

The compensatory movement was investigated by measuring the position of the basal segment with respect to the body at different positions of the body around the pitch axis. A small rigid wand was attached to the basal segment and a larger one to the anterior carapace of the animal. A telescope containing an angular graticule was focused on both wands and the angle between them measured. In this way, the angle of the basal segment to the animal was measured. The crab was held in a clamp and rotated in 10^{o} steps through 90^{o} head-up or head-down from the horizontal position. After each movement, several minutes were allowed before the position of the segment was measured.

Figure 4 shows the compensatory movement of the

basal segment as a percentage of the movement of the animal. The compensation is greatest over a narrow range either side of the zero position, and declines outside that range. The same curve was obtained for forward and backward pitch of the animal and no significant differences were observed between the responses of animals with or without visual input and with or without legs in contact with a substrate.

The function of this movement is unknown. If it is compensatory, it is not very efficient, having a maximum gain of about 0.2. Such rotation of the basal segment will alter the orientation of the thread hairs with respect to the vertical but not sufficiently to maintain the upper and lower groups in the vertical.

Physiological Responses of Thread Hairs to Gravity

The activity of thread hair units was recorded when the isolated, intact statocyst was tilted to different positions. The apparatus used was the same as that used to record the dynamic responses and, in fact, both types of analysis were often conducted on the same preparation.

If a recording is made from upper thread hair units as the statocyst is tilted slowly and smoothly out of its normal position through 90^{o} about the pitch or roll axis, the nervous activity gradually rises to a level in excess of 300 Hz or falls to zero. This is thought to be due to an extreme bending of the hair, under the influence of gravity, to a point where the nervous response is either saturated or blocked. If the new position of the statocyst is maintained, this extreme response of the receptors is also maintained, at least for 5 minutes or longer.

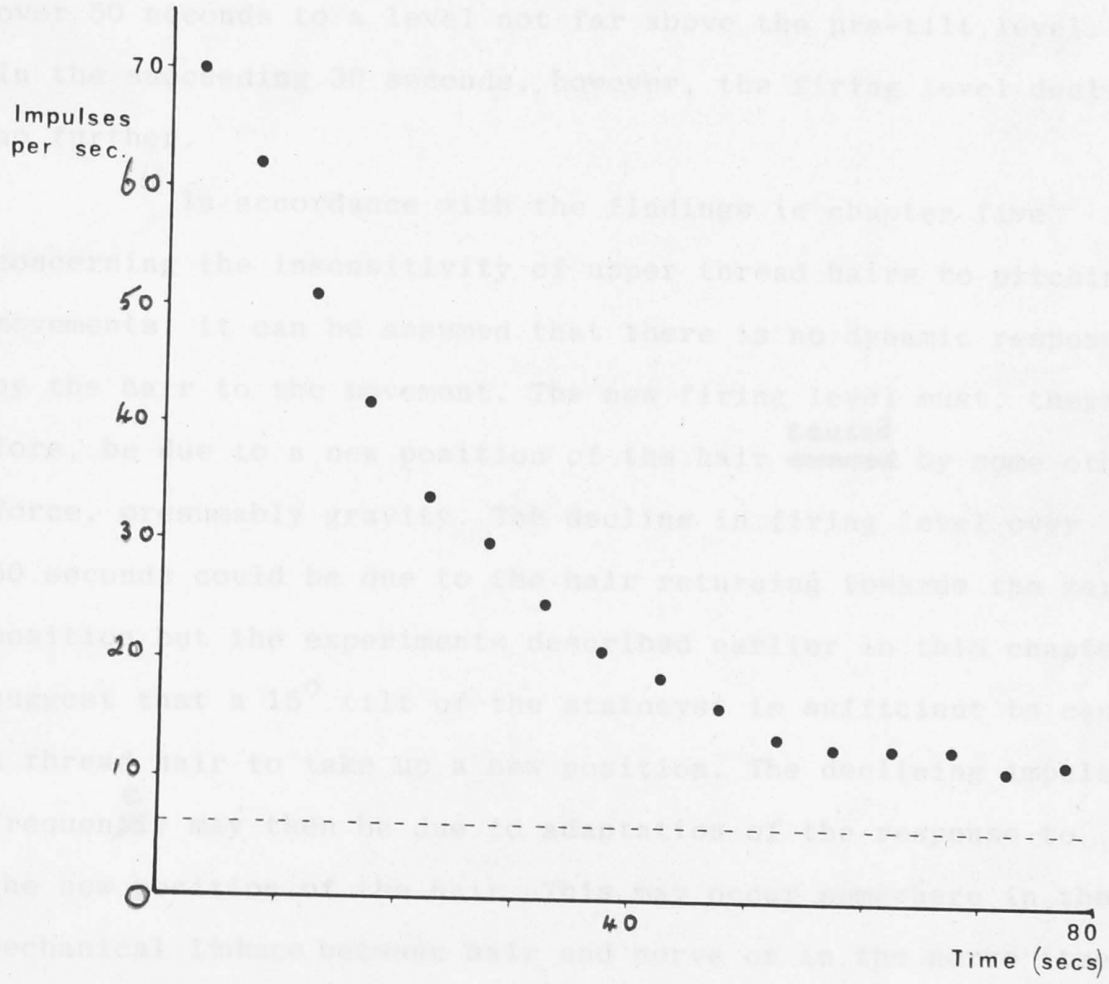


FIG. 5 Response of single thread hair unit to 15° tilt of the statocyst in a head-down direction. New position is assumed at zero and maintained. Broken line is pre-tilt impulse frequency.

A different response is obtained if the statocyst is tilted through a smaller angle. Fig. 5 shows the response of an upper thread hair unit to a new position of the statocyst 15^{o} head down from the normal. The firing frequency rises sharply when the new position is assumed and then declines over 50 seconds to a level not far above the pre-tilt level. In the succeeding 30 seconds, however, the firing level declines no further.

In accordance with the findings in chapter five concerning the insensitivity of upper thread hairs to pitching movements, it can be assumed that there is no dynamic response by the hair to the movement. The new firing level must, therefore, be due to a new position of the hair ~~cuased~~ caused by some other force, presumably gravity. The decline in firing level over 50 seconds could be due to the hair returning towards the zero position but the experiments described earlier in this chapter suggest that a 15^{o} tilt of the statocyst is sufficient to cause a thread hair to take up a new position. The declining impulse frequency may then be due to adaptation of the response to the new position of the hair. This may occur somewhere in the mechanical linkage between hair and nerve or in the nerve itself.

An interesting feature of the gravity response of thread hairs is the effect on the firing level when the statocyst is returned to the normal position after a prolonged tilt. Fig. 6 shows the results of an experiment in which the statocyst was tilted through 25^{o} in a head up direction in 5^{o} steps and then returned to zero. The impulse frequency of the thread hair unit was recorded every 10 seconds for the first 30 seconds and then every 30 seconds for about four minutes after each step of the tilt. The overall picture is of a decline in firing

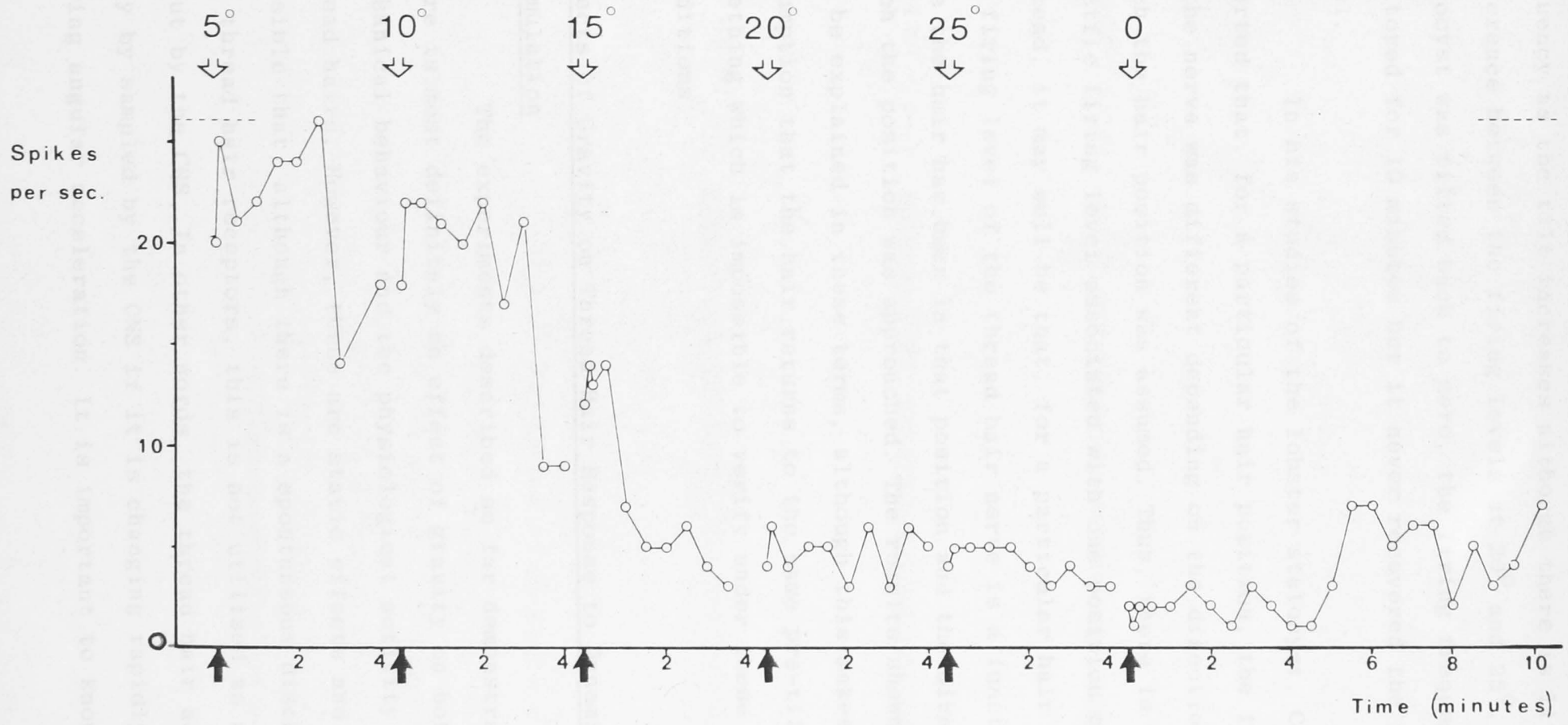


FIG.6 Response of single thread hair unit to 25° forward pitch in 5° steps and subsequent return to zero position. Broken line is pre-tilt impulse frequency.

frequency as the tilt increases although there is little difference between the firing levels at 20^{o} and 25^{o}. When the statocyst was tilted back to zero, the firing frequency was monitored for 10 minutes but it never recovered the pre-tilt level.

In his studies of the lobster statocyst, Cohen (1960) reported that, for a particular hair position, the firing level of the nerve was different depending on the direction from which the hair position was assumed. Thus, there is not one specific firing level associated with one position of the hair. Instead, it may well be that, for a particular hair position, the firing level of the thread hair nerve is a function of the time the hair has been in that position and the direction from which the position was approached. The results shown in Fig. 6 may be explained in these terms, although this makes the assumption that the hair returns to the same pre-tilt position, something which is impossible to verify under these experimental conditions.

Effects of Gravity on Thread Hair Response to Dynamic Stimulation

The experiments described so far demonstrate that there is most definitely an effect of gravity on both the mechanical behaviour and the physiological activity of the thread hairs. However, these are static effects and it is possible that, although there is a spontaneous discharge in the thread hair receptors, this is not utilised as a tonic input by the CNS. In other words, the thread hair activity may only by sampled by the CNS if it is changing rapidly, i.e. during angular acceleration. It is important to know, then,

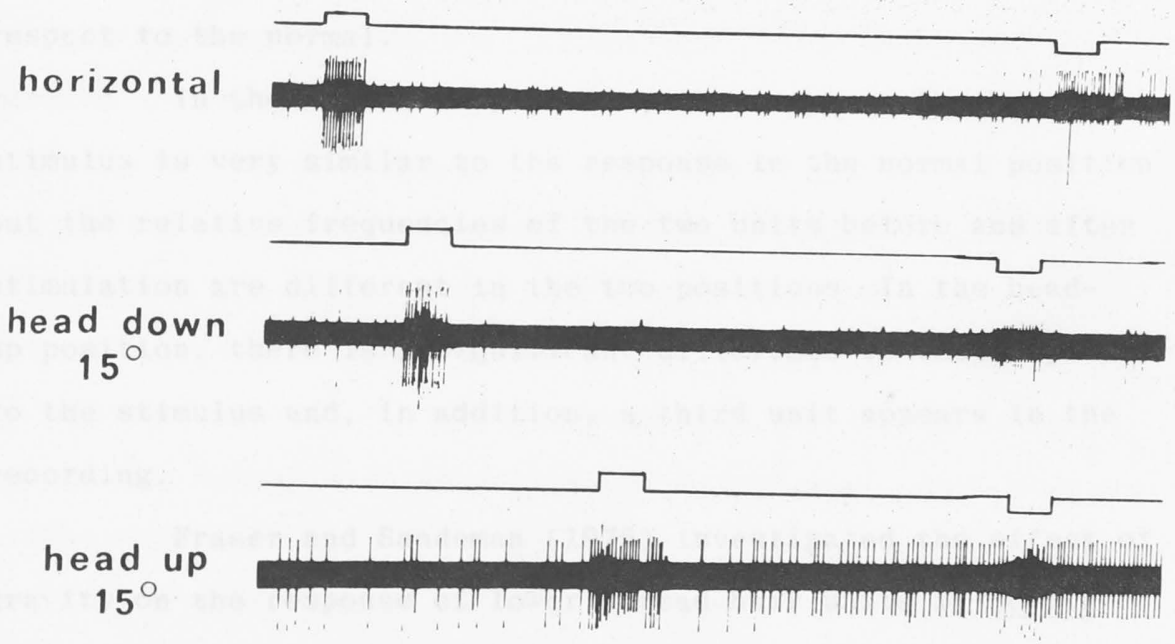


FIG. 7 Response of thread hair units to constant velocity rotation (40°/sec) in the plane of the horizontal canal at three different positions of the statocyst. Stimulus trace shows duration and direction of rotation.

1sec.

whether the observed static changes affect the responsiveness of the system to dynamic stimulation.

Fig. 7 shows the response of upper thread hair units to constant velocity rotation in the plane of the horizontal canal, at three different orientations of the statocyst. The experiment approximates to a situation in which the animal is rotated about its own vertical axis in its normal horizontal position and also in positions 15^{o} head-up or head-down with respect to the normal.

In the head-down position, the response to the stimulus is very similar to the response in the normal position but the relative frequencies of the two units before and after stimulation are different in the two positions. In the head-up position, there is a significant difference in the response to the stimulus and, in addition, a third unit appears in the recording.

Fraser and Sandeman (1975) investigated the effect of gravity on the response of lower thread hair units in Scylla to dynamic stimulation of the vertical canal. They found a significant difference in the response to a standard oscillatory stimulus depending on which side of the mean position the oscillation commenced. The important point about these experiments is that the responses were recorded from interneurons in the oesophageal connectives which are driven almost exclusively by thread hair units. Many primary afferents are assumed to converge on each interneuron so this finding implies that the effects of gravity on dynamic responses are not filtered out by averaging the responses of many primary receptors, but are conveyed to the CNS. Therefore, even if the positional responses of the thread hairs are not used to

monitor the animal's position in space, they may be used to modulate the responses to angular accelerations.

In Scylla, the thread hairs have been shown to be susceptible to gravity. They have been observed to change their position under the influence of gravity and a change in nervous activity has been recorded when the intact statocyst is tilted, an effect which is assumed to derive from an altered position of the hairs. Finally, the response to angular acceleration has been shown to depend on the orientation of the statocyst with respect to gravity. Whether the effect of gravity on nervous activity of the thread hairs is utilised by the animal for monitoring position is unknown. Dijkgraaf (1956) reported that, in Carcinus and Maja, if all the statocyst hairs except the thread hairs were denervated, all positional reflexes of the animal were abolished. This suggests that the thread hairs supply the animal with no positional information. That gravity affects the response of the thread hairs to angular acceleration is clearly demonstrated but how the animal interprets such responses is unknown. Perhaps a higher order interneuron receives inputs from static and dynamic receptors and assesses the responses of the latter in the light of information from the former.

In the lobster statocyst, Cohen (1955) has reported no effect of gravity on spontaneous activity or dynamic responses of thread hairs. His explanation for this, though it may, in fact, be correct, does not necessarily follow from his experimental results. Thus, having shown that the thread hair nerve did not fully adapt after more than 60 seconds, Cohen concluded that the apparent lack of response of thread hair units to gravity must be due to the hair maintaining the same

position, regardless of the orientation of the statocyst. In support of this, he referred to an experiment in which a thread hair was mechanically displaced by up to 90^{o} and released; it always resumed its original position within 20 seconds. If the hair has inherent elasticity, it would be expected to return to zero when the stimulus is removed. However, when a statocyst is tilted, the hair is subjected to a continuing stimulus, the linear acceleration due to gravity, and the hair may be forced to take up a new position where the force of gravity is in equilibrium with that due to the elasticity of the hair. In spite of this point, Cohen's conclusion that the insensitivity to gravity is due to an unchanged hair position does seem the only one possible and it may be that the lobster thread hairs are much more rigid than those of Scylla so that the elastic restoring force always exceeds the gravitational force.

Statolith Hairs

Statolith units were occasionally found when looking for thread hair units in bundles IIB_2 and IIC.

Nerves from the statolith hairs have a spontaneous firing level. If the statocyst is tilted and maintained at a new position, the impulse frequency increases or decreases to a new level and maintains that level with only slight adaptation (Fig. 8). For each position, over quite a wide range, the hairs have a different firing level and, if the frequency is plotted against position of the animal, it can be seen that each hair has a maximum frequency of response at a particular position of the animal (Fig. 9).

In one recording, containing a very large and a very small unit, the large unit responded to pitch of the statocyst but not to roll and the small unit showed the reverse

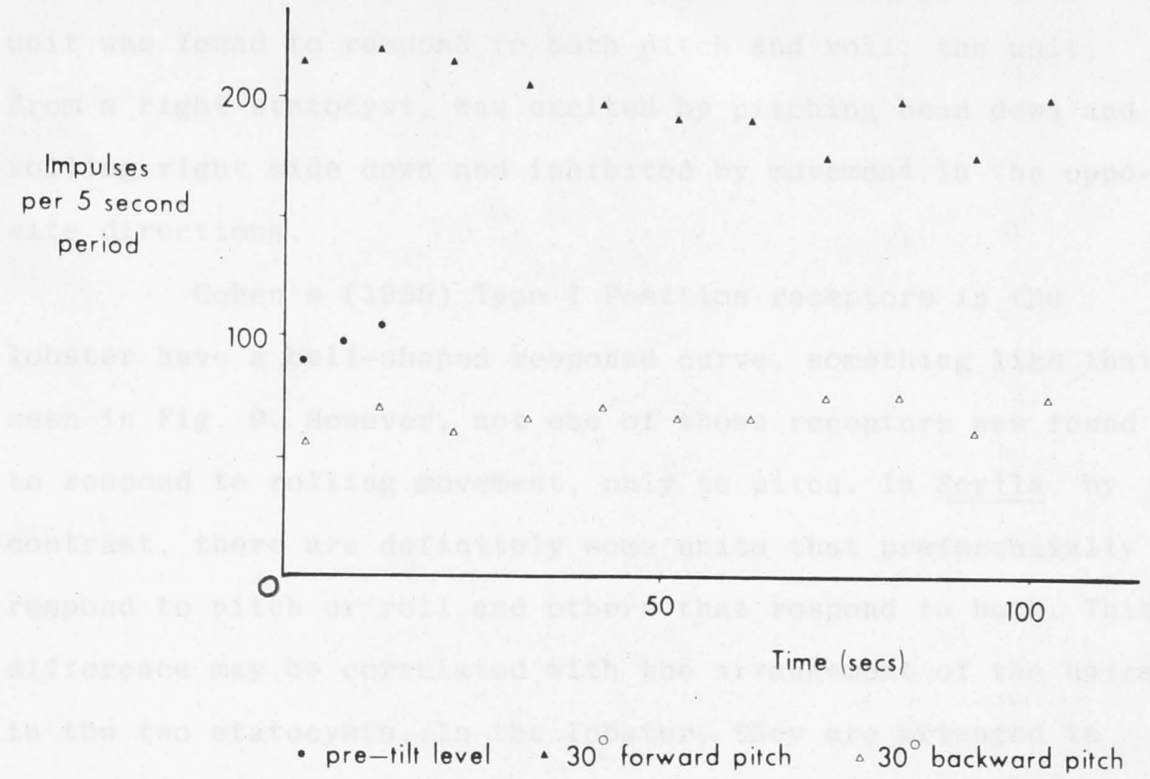


FIG. **8** Response of statolith unit to maintained positional change.

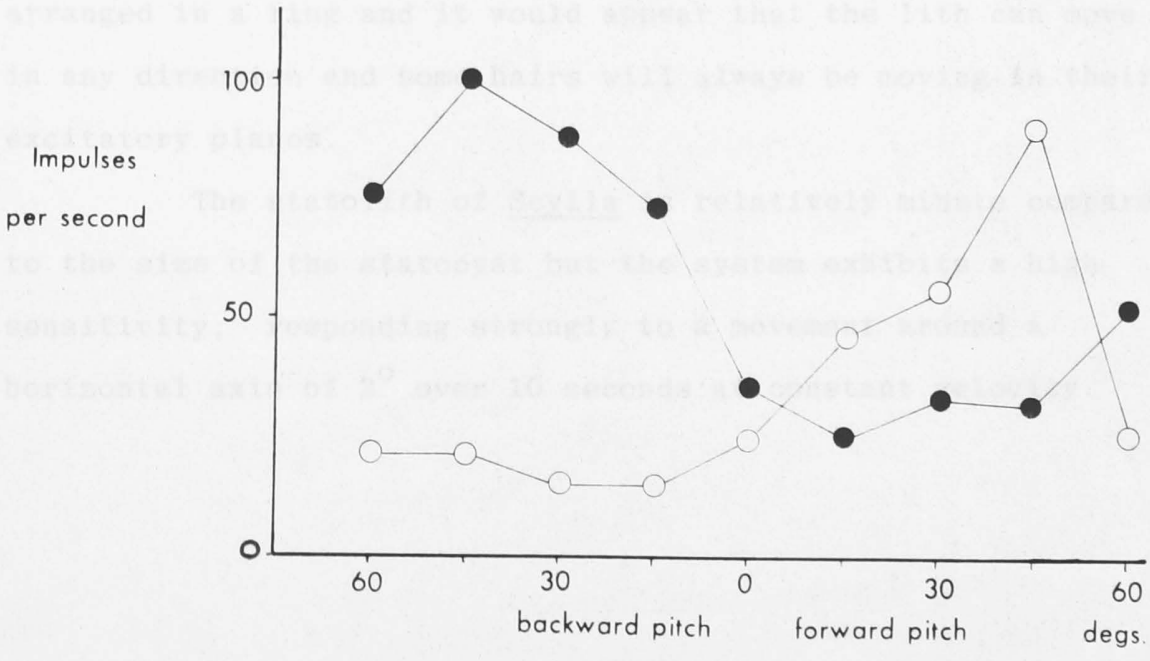


FIG. **9** Response of 2 statolith units to different positions about the pitch axis.

sensitivity. By contrast, in a different recording, a single unit was found to respond to both pitch and roll; the unit, from a right statocyst, was excited by pitching head down and rolling right side down and inhibited by movement in the opposite directions.

Cohen's (1955) Type I Position receptors in the lobster have a bell-shaped response curve, something like that seen in Fig. 9. However, not one of those receptors was found to respond to rolling movement, only to pitch. In Scylla, by contrast, there are definitely some units that preferentially respond to pitch or roll and others that respond to both. This difference may be correlated with the arrangement of the hairs in the two statocysts. In the lobster, they are arranged in straight or gently curved lines and it may be that only pitching movements cause any movement of the lith or any movement of the hairs in their excitatory plane. In Scylla, the hairs are arranged in a ring and it would appear that the lith can move in any direction and some hairs will always be moving in their excitatory planes.

The statolith of Scylla is relatively minute compared to the size of the statocyst but the system exhibits a high sensitivity, responding strongly to a movement around a horizontal axis of 2^{o} over 10 seconds at constant velocity.

CHAPTER SEVEN

Discussion

Introduction

The previous four chapters have described the statocyst of *Scylla* and detailed the structure and behaviour of the thread hairs. In summary, the statocyst is a complex cuticular sac that approximates to two hollow rings, or toroids, joined at rightangles. One ring is horizontal and one is vertical and, at the top and bottom of the vertical ring, there is a fine curtain of thread hairs across the canal. Each curtain consists of a single line of closely grouped hairs with lateral branches overlapping. The thread hairs are extremely sensitive to any movement of the fluid inside the statocyst. Because of the circular design of the statocyst, only angular acceleration will cause a directional fluid flow and the thread hairs are, therefore, primarily angular accelerometers.

The thread hairs project approximately perpendicularly from the surface of the sensory cushion. However, the exact angle of each hair to the cushion has been shown to depend on the orientation of the statocyst with respect to gravity. The ~~nerve~~ axon from a thread hair has a spontaneous impulse frequency which is a function of the position of the hair and the length of time it has been in that position; if the hair is bent, the impulse frequency either increases or decreases.

Transduction, Dual Innervation and Bidirectionality

When recording from single units, from either the upper or lower thread hairs, some are found to be excited by rotation in one direction while others are excited by rotation in the opposite direction (chapter five). It is known from the histology (chapter four) that each hair is supplied with two ~~nerves~~ sensory neurons and the suggestion was made that one ~~nerve~~ dendrite may be excited

by flexion of the hair one way while the other ~~nerve~~ is excited by flexion of the hair the opposite way. Whitear (1962) had proposed a similar arrangement for the two dendrites of the chordotonal scolopidia in the legs of Carcinus. In support of this arrangement in Scylla were the following facts. The two dendrites at the proximal end of a scolopidium often have different diameters and a survey of all the thread hair axons had indicated the presence of two populations of different diameters. As a physiological correlate, there was strong evidence of two populations of units, one giving large spikes and one small spikes, and there was tentative evidence of a correlation between spike amplitude and directional sensitivity (Silvey, Dunn, Sandeman; in press).

However, a detailed review of the evidence suggests that the paired dendrites of a scolopidium do not have opposite directional sensitivities. In 1965, Bush disproved Whitear's theory and, in 1967, Hartmann and Boettiger showed that movement cells paired within the same scolopidium responded to the same direction of movement. In reports since then (Young, 1970; Mill and Lowe, 1971, 1973), on different scolopidia, no evidence has been found that the two dendrites have opposite directional sensitivities.

In Scylla, the two dendrites are in contact at the proximal end of the scolopidium (Fig. 1). The junction closely resembles the ephapses reported in the scolopidia of the crab leg chordotonal organs (Whitear, 1962; Mill and Lowe, 1973). An ephapse is formed by the close apposition of the membranes of two nerves so that direct electrical interaction between the two is possible. The two nerves could only have opposite directional sensitivities if the electrical interaction was

Figure 1

Electron micrograph of proximal end of scolopidium showing the two dendrites (D) in contact with each other (arrow). The scolopale material (S) can be seen in the ~~inner sheath~~ scolopale cell and the ciliary roots (R) are prominent in each dendrite.

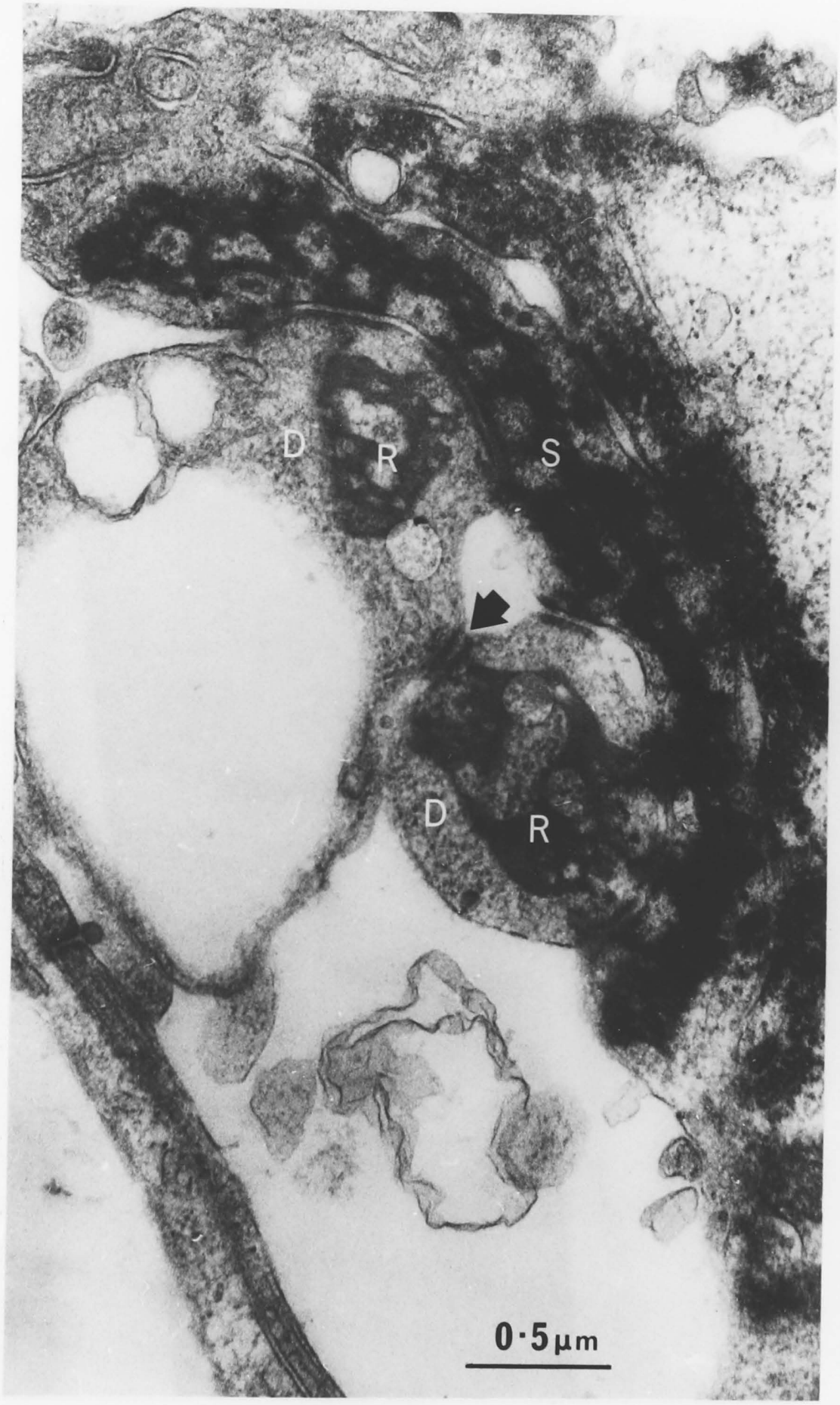


FIG. 1

mutually inhibitory, and that is a rare phenomenon, e.g. the Mauthner cells of the goldfish (Furukawa and Furshpan, 1963). There is physiological evidence in Scylla that the electrical interaction between the paired dendrites is not inhibitory. In one experiment, the responses from a single thread hair unit were being recorded during sinusoidal oscillation of the statocyst. A small unit was firing in response to rotation in one direction and becoming silent during rotation in the opposite direction. After several cycles of oscillation a second unit, four times the amplitude of the first, began firing (Fig. 2). However, every spike recorded from this large unit was superimposed on a small spike from the original unit. Not every small spike had a large spike superimposed but the large spike never occurrred alone. As accurately as could be measured, these two spikes were exactly synchronous and such synchrony strongly suggests coupling between neurons. There is no anatomical or physiological evidence of any coupling between the ~~nerves of~~ sensory neurons of different hairs and it is, therefore, suggested that the two units recorded are from the two dendrites of a single scolopidium. The electrical coupling is assumed to occur at the ephapse and the different spike amplitudes are probably correlated with the observed size difference between the paired dendrites and the two populations of different diameters in the axon bundle.

If the paired dendrites of a single scolopidium exhibit the same directional sensitivity, as suggested above, there are two questions to be answered: Why are there two dendrites to each hair, and why do some units, from a single population of hairs, respond to one direction of rotation and others to the opposite direction of rotation? To approach the second question first, Mill and Lowe (1973) investigated a similar situation in the PD proprioceptor of Cancer. The

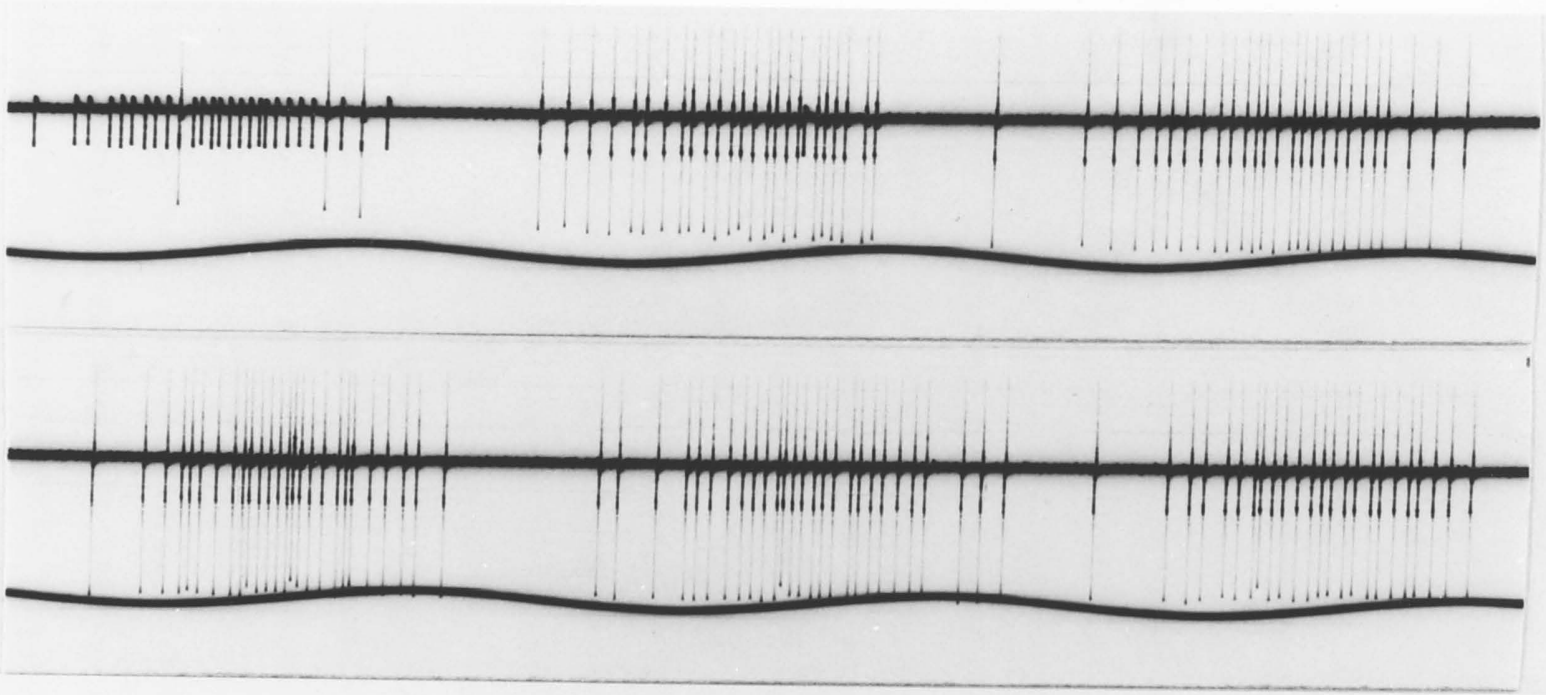


FIG. 2 Recording from upper thread hairs during sinusoidal oscillation about a vertical axis (1 Hz). Large unit appears during the oscillation but only occurs superimposed on the smaller one.

scolopidia are embedded in an elastic strand associated with one of the leg joints and some of the scolopidia are sensitive to elongation of the strand while others are sensitive to relaxation of the strand. Mill and Lowe showed that the elongation-sensitive and relaxation-sensitive scolopidia are embedded in different parts of the strand, which may partly explain the differential sensitivity, and they also showed that there are ultrastructural differences between the two types of scolopidia, which may also be correlated with their differential sensitivity. Each scolopidium contains a ciliary dendrite and a paraciliary dendrite and there are several structural features which tend to make the paraciliary dendrite the prominent component of elongation-sensitive scolopidia but the ciliary dendrite the prominent component of relaxation-sensitive scolopidia. These differences may be significant in terms of the directional response of the scolopidium but Mill and Lowe explained the difference in sensitivity mainly in terms of the tissue which surrounds the scolopidium in the strand. They proposed that longitudinal stretch is the adequate stimulus in both types of scolopidium. In an elongation-sensitive scolopidium the dendrites are held firm by their desmosomal attachment to enveloping cells, themselves firmly embedded in the strand. Elongation of the strand stretches the distal components of the scolopidium away from the dendrites. A relaxation-sensitive scolopidium is always found embedded in collagen, which is attached to the enveloping cells of the scolopidium at certain contact points. It is proposed that the collagen fibrils, oriented parallel to the long axis of the scolopidium, would resist longitudinal stretch during elongation of the strand. During relaxation of the strand, however, energy released by the collagen fibrils is thought to have an effect on the scolopidium through the

contact zones, resulting in a movement of the distal region away from the fixed dendritic region.

In Scylla there are no obvious differences between the two dendrites of a scolopidium. In this respect, the structure is more like that of the cockroach chordotonal organ described by Young (1970). No two scolopidia in Scylla ever look exactly the same in transverse section but there is no obvious division into types. They are all enveloped by several ~~sheath~~ enveloping cells, two distinct, large nuclei commonly appearing in a single transverse section. The tissue around the ~~sheath~~ enveloping cells appears to be homogenous. Despite this, there is the possibility that the scolopidia of one group of hairs are divided into two classes of opposite directional sensitivities. The structural basis for the directionality may lie in a feature of the surrounding tissue which has been overlooked or it may be associated with a more distal part of the system, the insertion of the chorda at the hair base, for instance.

The other unanswered question about the thread hair transduction system, and other similar scolopidial structures, concerns the number of dendrites associated with each scolopidium. This number is far from consistent; Young (1970, 1973) has reported two instances of single innervation and Schöne and Steinbrecht (1968) reported triple innervation. However, the most common number of dendrites seems to be two. As described earlier (Mill and Lowe, 1973), the two dendrites of some systems show marked differences in structure but those of Scylla do not, except that one usually has a greater diameter than the other. Even if the synchronous spikes described earlier (Fig. 2) are derived from the paired dendrites of one scolopidium, the experiment reveals very little about the possible relationship between the two. The small spike was present continuously and

showed the usual approximately sinusoidal variation in frequency during oscillation at frequencies from 0.25 Hz to 2.5 Hz. The large spike occurred at some time at each frequency of oscillation and always superimposed on the small spike. However, it did not maintain the same consistency as the small spike, often firing for two cycles and then missing one cycle or, as in Figure 2, first appearing only after several cycles of oscillation.

Schöne and Steinbrecht (1968) found three dendrites in each scolopidium associated with the statolith hairs of the crayfish and suggested that each may have a different sensitivity range or that each may be associated with a different mode of the response, phasic, phasic-tonic and tonic. Either of these explanations could apply to Scylla. For example, the lack of consistency of the large unit in the records may arise because it is at the very bottom of its sensitivity range. It is not known how the two dendrites could interact to produce a phasic and a tonic component to the response but the possibility cannot be discounted. Differences in the mechanical linkages of the two dendrites or differences in the membrane properties of the individual dendrites could make one dendrite phasic and one tonic. However, in Scylla, what evidence there is for a bicomponent response has come from recordings of single units of the primary sensory nerve, for example, the high frequency burst at the start of constant velocity rotation (chapter five). A recording from both axons of a scolopidium, intracellularly if possible, would provide the critical information on this point and might also throw further light on the information-coding capabilities of scolopidial organs in a wide variety of other situations.

The fundamental problem in sensory physiology is transduction of the stimulus, be it mechanical, chemical or optical, into nervous activity. In very few, if any, receptor systems is the exact transduction mechanism understood and, until it is, many peripheral problems such as directionality of response and dual or triple innervation can never be fully solved.

In many scolopidial systems the adequate stimulus is thought to be longitudinal stretch. Thus, Schöne and Steinbrecht (1968), Young (1970) and Mill and Lowe (1973) all concluded that longitudinal stretch was the adequate stimulus in their various studies. The dendrites are thought to be attached firmly to the enveloping cells by desmosomal contacts to provide a counterforce for stretch of the microtubular apparatus in a distal direction. All nerves can act as stretch receptors, that is, produce electrical changes in response to mechanical deformation of the nerve membrane. Longitudinal stretch of the microtubular apparatus of a scolopidium, therefore, is thought to stretch the membrane at the distal tip of the dendrite via the cilium firmly rooted in the dendrite. However, the exact mechanism has yet to be described in any system.

In Scylla there is no significant feature of the scolopidium which would preclude longitudinal stretch as the adequate stimulus. The system most resembles that described by Schöne and Steinbrecht (1968) for the statolith hairs of the crayfish. The mechanical stimulus is transmitted from the hair to the dendrites by the chorda which, in Scylla, consists of a collection of longitudinally oriented microtubules bound together by electron-dense material. In the crayfish the chorda is inserted at the hair base in such a way that bending of the hair would tend to stretch the chorda. The stretch of the dendritic membrane, therefore, is caused by longitudinal stretch

of the chorda transmitted down through the cilium. The difference in Scylla is that the chorda is not inserted at the hair base in a way which would result in stretch when the hair bends. Instead, it is attached to the thickened walls of a narrow neck at the base of the hair shaft in a manner which might result in lateral compression of the chorda when the hair bends. The insertion resembles that in the hair-plate receptor of the honey bee, as described by Thurm (1964, 1965). In that system the nerve gives rise to a cilium which develops into a "tubular body", longitudinally oriented microtubules in an electron-dense matrix. The neck of the hair is filled with a spongy material and the tubular body is embedded in the spongy material. Thurm showed that longitudinal stretch or compression of the tubular body evoked no nervous response but that lateral compression was the adequate stimulus, and he further showed how bending of the hair constricted the neck and caused such lateral compression.

Thurm made no suggestions as to how lateral compression of the tubular body might be transmitted to the dendritic membrane. Stretch of the membrane may still play a part in Scylla, the honey bee, or both. Thus, lateral compression of the chorda may cause longitudinal movement or elongation of microtubules within the chorda which could affect the dendritic membrane in the same way as longitudinal movement of the whole chorda. An alternative possibility, based on a proposal by Moran and Varela (1971) for the cockroach campaniform sensillum, is that compression of the microtubules in the chorda causes release of bound ions from the tubules themselves or from the matrix. Alteration of ion balance within the scolopidium might favour local current flow across the plasma membrane of the dendrite.

Thurm suggested that lateral compression may actually be the adequate stimulus even in those scolopidia where longitudinal stretch is thought to be so. Many scolopidia are characterised by a shape that tapers distally. Thurm suggested that longitudinal stretch might cause an increased tapering which would result in lateral compression of the microtubular components in the centre. The scolopidial structures show such a basic similarity in design that a unifying theory of this sort, allowing for interspecific modifications, would seem more appropriate than a series of disparate theories.

Comparative Physiology of Equilibrium Organs

In the vertebrate labyrinth the semicircular canals are specialised for the detection of angular acceleration, although a susceptibility to linear acceleration has also been demonstrated (Lowenstein, 1972). The sensory hairs are embedded in a gelatinous wedge that fills the entire cross-section of the canal. This is the cupula and it is attached at its base to the hairs but is otherwise free to move. Because the canal is circular, only angular acceleration in the plane of the canal will cause a directional fluid flow and, when this occurs, the cupula is forced by the fluid to bend about its point of attachment. In all vertebrates, except cyclostomes, there are three semicircular canals on each side of the head and they are arranged orthogonally, i.e. one horizontally and two vertically at rightangles to each other. Rotation about one of the three major axes, therefore, will stimulate mainly the canal in the plane of the rotation. Rotation about axes other than these will stimulate two or three canals to different degrees and the combined input from them enables the CNS to compute the magnitude and direction of the rotation.

In Octopus vulgaris there is a statocyst of considerable complexity, capable of detecting linear and angular accelerations (Young, 1960; Dijkgraaf, 1961; Barber, 1966; Budelmann et al, 1973). There is no canalisation of the statocyst but the animal can discriminate between angular accelerations around the three major axes. The receptors responsible for this discrimination are found on three cristae arranged orthogonally; each crista bears a patch of fine hairs embedded in a cupula. Canalisation is thus not essential for detection of angular acceleration, although the internal architecture of the octopus statocyst in the region of the cristae may be designed to direct fluid flows in a particular direction during such stimulation.

The statocyst of the crab, like the labyrinth of the cyclostome, has only two semicircular canals. The degree of canalisation of the crab statocyst is variable. Carcinus has been reported to have a canalicular structure for many years (see Hensen, 1863) but Fraser states that the canals in Carcinus are much less well-defined than those of Scylla (personal communication). Leptograpsus, the rock crab, also has a canalicular statocyst but, again, the canals are less pronounced than in Scylla (personal observation).

In chapter five it was shown that the thread hairs of Scylla are functionally divided into an upper and a lower group and that the upper group respond to rotation in the plane of the horizontal canal while the lower group respond to rotation in the vertical plane. The crab statocyst thus has one canal to detect angular acceleration about a vertical axis and one canal to detect angular acceleration about both horizontal axes. In terms of the animal, the vertical canal is responsible for detecting both pitching and rolling movements. To fulfil this

function, the plane of the vertical canal is at an angle of 45^{o} to both the longitudinal and transverse axes of the animal. Fraser and Sandeman (1975) showed that the optimum response of the lower thread hairs was obtained during rotation in the plane of the vertical canal, as would be expected, but that the hairs still responded if rotation was in a vertical plane at 45^{o} to the plane of the vertical canal. Pitch or roll of the animal, therefore, will both stimulate the vertical canal, although neither involves rotation in the plane of that canal. In this way the animal compensates for not having three orthogonal çanals but it is a compromise solution, for two reasons. Rotation in the vertical plane is mostly around the pitch or roll axes, i.e. 45^{o} to the plane of the vertical canal, with the result that the vertical canal is rarely rotated in its optimum plane. Secondly, because the vertical canal responds to both pitch and roll, the information from a single statocyst can never tell the animal whether it is, in fact, pitching or rolling. The vertical canals of an animal's two statocysts are at rightangles to each other and Sandeman and Okajima (1972) suggested that the ambiguity inherent in the response of a single statocyst to pitch or roll could be resolved by comparing the repsonse with that from the contralateral statocyst. Fraser and Sandeman (1975) later investigated directional interneurons in the oesophageal connectives and showed how the outputs of the interneurons on either side could be added to give the pitch component of rotation or subtracted to give the roll component. Using the output of both statocysts, therefore, the animal can discriminate angular accelerations about any axis.

The vertebrate semicircular canal has been extensively investigated in every group from cyclostomes to man. When the canal is rotated the enclosed fluid is temporarily left behind, due to its inertia. This causes, effectively, a fluid flow in the direction opposite to that of the rotation and this fluid flow causes the cupula to bend about its point of attachment. Embedded in the cupula are sensory hairs and these are caused to bend with the cupula. The bending of the hairs has a depolarising or hyperpolarising effect on the sensory cell from which they arise and this electrical change is transmitted synaptically from the sensory cell to the afferent nerves.

Steinhausen (1931) observed the behaviour of the cupula of the pike semicircular canal during angular acceleration. He noted that the cupula fitted tightly in the ampulla of the semicircular canal, and also that the cupula had an inherent elasticity which caused it to return to its mean position, over a certain time, in the absence of any angular acceleration or deceleration, for instance, during rotation at constant velocity. As a result of his observations and experiments Steinhausen concluded that the cupula in the endolymph-filled canal functions as an overdamped torsion pendulum. As a consequence, the semicircular canal acts as an inertial angular accelerometer whose behaviour, in terms of its responses both to sinusoidal and to stepwise stimulation, satisfies a second-order differential equation such as:

$$XA'' + YA' + ZA = 0$$

where X = moment of inertia

Y = moment of friction

Z = cupula restoring couple

A'' = angular accleration...
A' = angular velocity.....
A = angular displacement..
} of the cupula-endolymph system relative to the skull

Steinhausen thought that the main frictional component was that generated between the cupula and the viscous endolymph, but Steer (1967) demonstrated that there was also appreciable friction between the cupula and the wall of the ampulla. The response time of the system to an imposed acceleration is determined by a combination of the inertia (X) of the fluid and the friction (Y). The recovery time of the system, on the other hand, is determined by a combination of the friction (Y) and the elasticity of the cupula (Z). The system can thus be characterised by its two time constants, the short one given by X/Y, and the long one given by Y/Z. Both have been measured experimentally in some vertebrates, the longer one being between 10 and 30 seconds (Lowenstein, 1972), and the shorter one about 50 milliseconds (Van Egmond et al, 1949). However, theoretical calculations of the shorter time constant, based on dimensions of the canals, viscosity of the endolymph and other parameters, give a value that is smaller by about one order of magnitude (Young, 1969). This suggests that, although considerable progress has been made, the semicircular canals can still not be modelled accurately.

From the torsion pendulum model the behaviour of the cupula-endolymph system can be predicted during sinusoidal oscillation. Thus, it is predicted that the displacement of the cupula is in phase with the acceleration at very low frequencies, in phase with the velocity at the natural frequency, and in phase with the displacement of the canal at very high frequencies. When the phase of the response is measured

at different frequencies of stimulation, either by observing the cupula or by recording from the primary afferent nerves, this is found to be the case. Over a certain frequency range, often spanning two log units of frequency, the response is approximately in phase with the velocity (see Jones, 1971). There is only one natural frequency within that range but the hydrodynamics of the canal, especially the damping, are such that the cupula is in phase with the velocity over this expanded range of frequencies. Jones and Spells (1963) showed that the frequency range over which the velocity is monitored varies in animals of different sizes but that the range always coincides with the frequency of head movements encountered by an animal of that size. They showed that a big difference in body weight is correlated with only a small difference in canal dimensions but that that small difference is sufficient to alter the hydrodynamics of the canal and hence set the frequency range over which velocity is monitored.

Fernandez and Goldberg (1971) conducted a detailed investigation of the responses of the semicircular canal of the squirrel monkey during sinusoidal stimulation. They found that the response deviated slightly from that predicted by the torsion pendulum model. They found a phase lead component over the central range of frequencies such that the response was never exactly in phase with the velocity but always ahead of it. The phase lead of the response decreased as the stimulation frequency increased, up to 0.5-1 Hz. It came within 10^{o} of the velocity but then, as the frequency was further increased, the phase lead began to increase again instead of becoming a phase lag. A possible explanation was proposed for the response but it was largely speculative and only served to emphasise that there is much to be learnt about the dynamic response of the

semicircular canal.

The thread hairs in the crab statocyst are structurally different in many ways from the vertebrate semicircular canal and yet there seems to be a fundamental similarity. The fluid-filled canals of the statocyst undoubtedly function as accelerometers and, as experiments in chapter five showed, they perform one integration so that the position of the fluid and, therefore, the hairs, is in phase with the velocity. As in the vertebrates, there is a range of frequencies of sinusoidal stimulation over which the response is approximately in phase with the velocity and this range coincides with the natural range of movements experienced by the animal. At higher frequencies, the response lags the velocity, as predicted by the torsion-pendulum model, but contrary to the findings of Fernandez and Goldberg described above.

Physically, the canal diameter in the statocyst is more than double that in, for instance, the human semicircular canal (Jones, 1971). The statolymph does not have a high viscosity, unlike vertebrate endolymph, so that frictional forces are assumed to be much less important. The thread hairs form a curtain across the canal but no cupula has ever been reported and the hairs do not appear to stretch as far as the opposite wall of the canal. The elasticity of the thread hairs of Scylla has not been measured directly, although Cohen (1955) showed that the thread hairs of the lobster take only 0.5-1 sec. to return to the spontaneous level of activity following strong stimulation. In chapter five, the impulse frequency of the thread hair units in Scylla was seen to return to the spontaneous level after 1 second of constant velocity rotation. In chapter six,

it was shown that the sensory adaptation for a hair displaced from its mean position is incomplete even after 60 secs, so the return of the impulse frequency to the spontaneous level during constant velocity rotation is assumed to be due to the return of the hair and not to sensory adaptation. The time taken for the hair to return to its resting position is assumed to be a function of the displacement, which is, in turn, a function of the strength of the stimulus, as is the case for the vertebrate cupula (Steinhausen, 1931). The rotation described for Scylla was only 10^{o}/sec so that the hair may not have been displaced very much. However, the statocyst was rotated at constant velocities up to 60^{o}/sec and, though it was not possible to monitor the impulse frequency during prolonged rotation at these speeds, the response to a sudden stop could be measured. The rotation was stopped after different lengths of time at each speed because of the design of the equipment, with the result that the hairs in each experiment would be displaced by different amounts at the time the rotation stopped. The sudden deceleration would thus cause the hairs to overshoot by different amounts and yet, in all experiments, the spontaneous level was recovered within 1 second. This does not provide a direct measurement but does suggest that the thread hairs revert to their resting position within 1 second of the onset or sudden cessation of constant velocity rotation, at least up to speeds of 60^{o}/sec.

Although the thread hair-statocyst system appears to function on the same principle as the cupula-endolymph system, even to the point of behaving as a torsion pendulum, perhaps, it is very unlikely that the differential equation derived for the vertebrate system can be applied to the crab system

without modification. The canals in the statocyst are not closed like the semicircular canals and the dimensions along their length are far from uniform. In addition, the damping of the cupula is dependent on the high viscosity of the enclosed fluid, accentuated by the narrow canals, and the tight fit of the cupula in the canal, and none of these features are present in the statocyst. Although the statocyst is superficially more complex in shape than a semicircular canal, it may be a more primitive angular accelerometer. As such, it may turn out to be easier to model its behaviour than has proved to be the case for the semicircular canal.

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